St. Edmund's College

(Affiliated to North Eastern Hill University, Shillong)
Recognized by the University Grant Commission under 2 (f) and 12 (B) of UGC act 1956)
Laitumkhrah, Shillong – 793003, Meghalaya, India

E-mail: stedmundscollege@gmail.com Website: http://sec.edu.in

TO WHOM IT MAY CONCERN

This is to certify that the following number of students of Biotechnology honours have completed the Internship Programme that was organized by the Biotechnology department for the year 2021-22.

Roll No	Name of the student	Dates	Duration	Place of Training
S2001339	Akriti Prasad	10th July 2022- to 25th July 2022	15 Days	Dr Akule Puro Division of Animal Health ICAR Research Complex for NEH region
S2001342	Rohini Gautam	10th July 2022- to 25th July 2022	15 Days	Dr Akule Puro Division of Animal Health ICAR Research Complex for NEH region
S2001345	Manash Jyoti Das	10th July 2022- to 25th July 2022	15 Days	Dr Akule Puro Division of Animal Health ICAR Research Complex for NEH region
S2001337	Barsha Rani Bora	10th July 2022- to 25th July 2022	15 Days	Department of Bioengineering IIT- Guwahati
S2001344	Dorin Pathak	10th July 2022- to 25th July 2022	15 Days	Department of Bioengineering IIT- Guwahati
S2001325	Anindita Mudiar	10th July 2022- to 25th July 2022	15 Days	Dr Sapta Rishi Paul Epygen Biotech Ltd Navi Mumbai
S2001326	Antarleena Bhattacharjee	10th July 2022- to 25th July 2022	15 Days	Dr Sapta Rishi Paul Epygen Biotech Ltd Navi Mumbai
S2001324	Mridushmita Das	3rd July 2022 onwards	15 Days	Mr K J Nongkynrih Department of Biotechnology St. Edmund's College
S2001336	Abantika Goswami	10th July 2022- to 25th July 2022	15 Days	Mr K J Nongkynrih Department of Biotechnology St. Edmund's College
S2001334	Rebecca D L Marbaniang	5th August – 20th August 2022	15 Days	Dr Deepika Singh Advanced Analytical Instrumentation Lab, IIIM – Jammu [CSIR]
S2001340	Vaishali Nayak	5th August – 20th August 2022	15 Days	Dr Deepika Singh Advanced Analytical Instrumentation Lab IIIM – Jammu [CSIR]
S2001333	Jasmine Jamatia	10th July 2022- to 25th July 2022	15 Days	Dr Viki Manners Department of Silviculture Govt. of Meghalaya

S20001338	Suman Basumatary	10th July 2022- to 25th July 2022	15 Days	Dr Viki Manners Department of Silviculture Govt. of Meghalaya
S20001339	Phidarikynti Thangkhiew	10th July 2022- to 25th July 2022	15 Days	Dr Viki Manners Department of Silviculture Govt. of Meghalaya
S2001330	Pynbianglang Dkhar	10th July 2022- to 25th July 2022	15 Days	Dr Viki Manners Department of Silviculture Govt. of Meghalaya
S2001332	Cassiana Poshna	10th July 2022- to 25th July 2022	15 Days	Dr Viki Manners Department of Silviculture Govt. of Meghalaya
S2001322	Sanjida R Choudhury	10th July 2022- to 25th July 2022	15 Days	Department of Genetic Engineering Shah Jalal University of Science & Technology, Sylhet, Bangaladesh

(Principal)

EPYGEN BIOTECH PRIVATE LIMITED

INTERNSHIP REPORT

DURATION: 21st July to 4th August, 2022

LOCATION: N 28/2, Addl. Patalganga

MAHARASHTRA 410222, INDIA

SUBMITTED BY:

ANINDITA MUDIAR

(BSC 5th SEM

ST EDMUNDS COLLEGE)

SUBMITTED TO:

DR SAMRAT ADHIKARI

HOD BIOTECHNOLOGY

ST EDMUNDS COLLEGE

CERTIFICATE

Epygen Biotech Pvt. Ltd
N 28/2, Patalganga, Maharashtra 410220, India.

Certificate of Participation

Awarded to ***Ms. Anindita Mudiar,*** *student of Vth Semester of B.Sc., Biotechnology at St. Edmund's College, Shillong, for actively participating in 15 Days Summer Training w.e.f 21st July, 2022 to 5th August 2022 on* ***"Recombinant Protein Expression"*** *that comprised of Media preparation, Culturing of fungal cells in solid and liquid media, cell harvesting, Protein and Glucose estimation, SDS PAGE and Western blotting at Epygen Laboratories under the guidance of Dr S. Paul, Lead Scientist, Epygen Biotech Pvt. Ltd., Mumbai. This programme was organized by Epygen Biotech Pvt. Ltd and Biotechnology Department of St Edmund's College, Shillong.*

Debayan Ghosh
Chairman,
Epygen Biotech

EPYGEN BIOTECH PRIVATE LIMITED * MUMBAI *

epygen

CONTENTS

PREFACE AND ACKNOLEDGEMENTS

From 21st July to 4th August, 2022, I had the privilege and pleasure of joining a team of dedicated scientists at EPYGEN BIOTEH PVT LIMITED, Patalganaga. I was given the opportunity to learn on the topic " RECOMBINANT DNA TECHNOLOGY". The main motive and goal of the internship was to gain practical knowledge and hands- on training on various techniques associated with the topic.

The 15 days long internship period has not only given me the knowledge on the specific topic but also has enlightened me with the exposure to handle instruments and analysis of macromolecules at an industrial level.

I would specifically like to acknowledge and extend our sincere thanks to Dr. Saptarishi Paul, leading scientist at EPYGEN and other assisted scientist who have extended their helping hands throughout the internship. I would like to thank to Mr. Debayan Ghosh, President and founder Epygen group,Dubai,USA,India, for allowing me to join their renowned company as intern. I would like to extend my deepest gratitude to Dr Samrat Adhikari, HOD Biotechnology dept, St Edmunds College and Dr Sylvanus Lamare, Principal, St Edmunds College for believing in me and providing with the opportunity to have my internship from EPYGEN BIOTEH PVT LMT.

MEDIA PREPARATION

(LIQUID/SOLID)

Cultural media is the substance that is required for the growth of microorganisms. Microorganism use this media as their food, containing all the required nutrients when they grow invitro. Cultural media very in their form and composition determined by the species to be cultivated.

Cultural media can be liquid or solid depending on the addition of Agar. Agar is the solidifying agent consisting of polysaccharide.

STEPS:

1. Place a glass beaker containing required quantity of distilled water over a spinning machine (Vortexing). A rod shaped magnet (magnetic flea) is placed in the beaker which produces a magnetic field and in turn helps to mix the components properly.
2. All the required components are added one after another into the beaker after proper weighing on a weighing machine.
3. Once the components are mixed thoroughly with the distilled water, agar is added if a solid cultural media is required. (NOTE: Agar is added at the end)
4. The PH of the media prepared is measured with a PH meter and adjusted using a base (NaOH) or acid (HCL).
5. The media is then poured into a conical flask and covered with cotton plug followed by wrapping with a foil paper.
6. The media is then autoclaved.

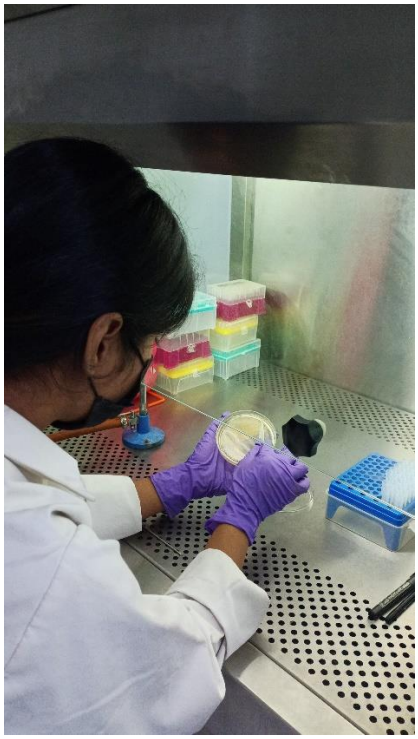
PREPARATION OF AGAR PLATES

1. Petri dishes, media prepared and other equipments are placed under UV radiation in the laminar flow for sterilization.
2. Other stock solutions are mixed with the media.
3. Some volume of the media are poured into the petri dishes. There should not be any bubble in the dishes.
4. The plates are then stored.

STREAKING METHOD

Streaking means " a long, thin line. Streaking plate method is a microbiological culture technique where a sample is spread in a petri dish in the form of a long, thin line over the surface of solid media.

(NOTE: Do not pierce the media while streaking)



RESULT AND OBSERVATION

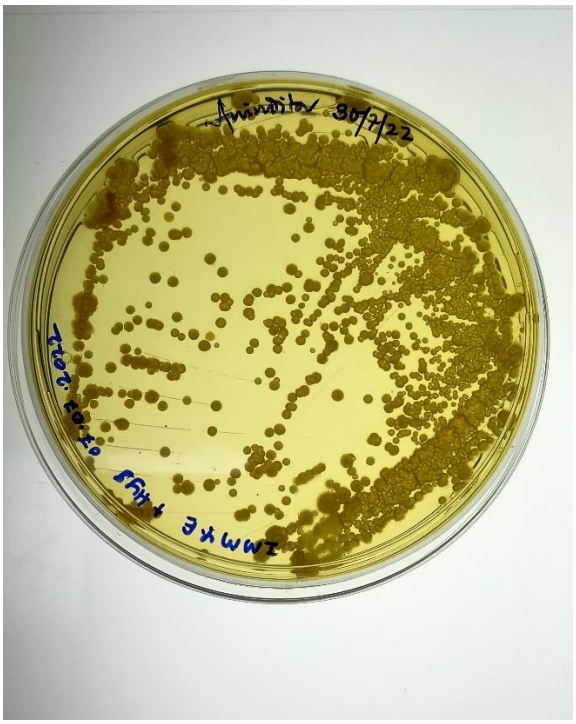


Fig: Growth observed after 3 days from streaking

Protein Estimation by Bradford-Method

Principle-

The Bradford protein assay is used to measure the concentration of total protein in a sample. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue. This method actually measures the presence of basic amino acid residues, arginine, lysine and histidine, which contributes to formation of the protein-dye complex. It is based on an absorbance shift of the dye Coomassie Brilliant Blue-G250. When the dye binds to the protein, it causes a shift from 465 nm to 595 nm, which is why the absorbance readings are taken at 595 nm. The increase of absorbance at 595 nm is proportional to the amount of bound dye, and thus to the amount (concentration) of protein present in the sample. If there's no protein to bind, then the solution will remain brown.

Materials/Reagents Needed-

1. Bovine Serum Albumin (BSA)
2. Coomassie Brilliant Blue G-250
3. Methanol
4. Phosphoric acid (H_3PO_4)
5. Bradford reagent

Equipment-

Spectrophotometer

Method-

Bradford method

Procedure-

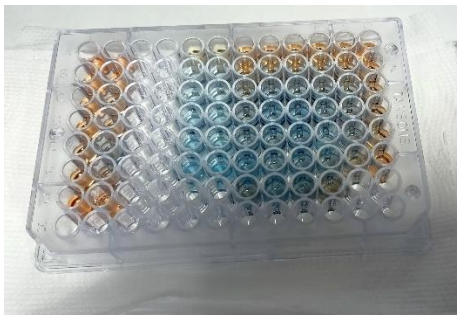
A. Standard assay procedure (for sample with 0.2-1.0 mg/ml of protein)

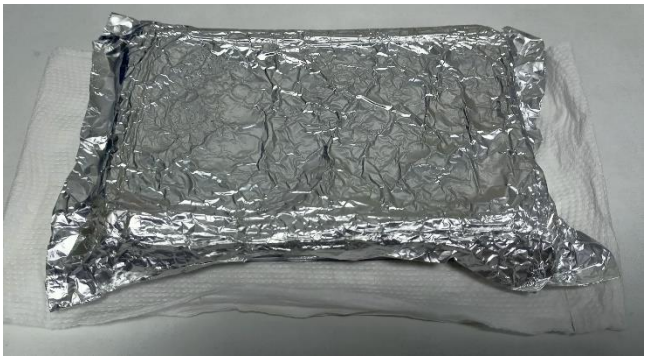
1. Prepared five dilutions of a protein (usually BSA) standard with a range of 0.2 mg to 1 mg protein.

2. Diluted unknown protein samples to obtain 0.2-1.0 mg protein/ml.
3. Added 10 µl each of standard solution or unknown protein sample to an appropriately labelled test tube.
4. Set two blank tubes. For the standard curve, added 10 µl H_2O instead of the standard solution. For the unknown protein samples, added 10 µl protein preparation buffer instead. (*Note: Protein solutions are normally assayed in duplicates or triplicates*).

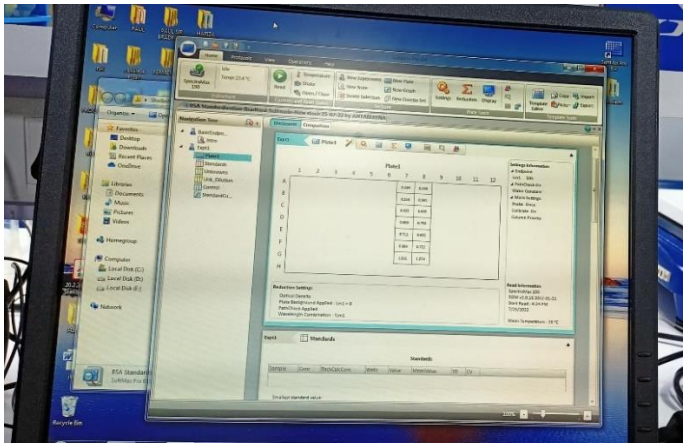
5. Added 240 µl of Bradford reagent to each tube and mix well.

6. Incubated at room temperature (RT) for 10 min. (Note: *Absorbance increases over time; samples should be incubated at RT for no more than 15 minutes)* .
7. Measured absorbance at 595 nm





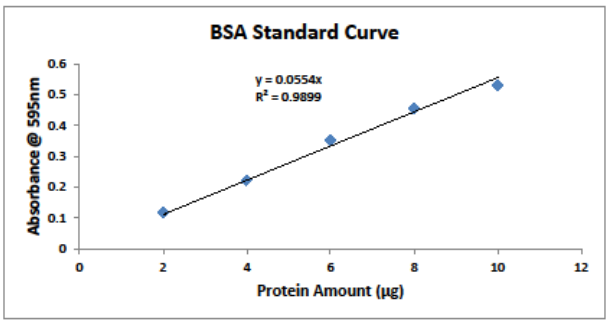
Observation and Inference-

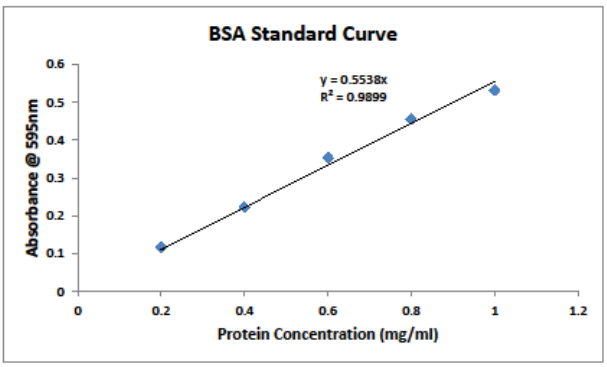


BSA STANDARDIZATION OF NEW BRADFORD REAGENT

In each well = 240 µL Bradford + 10 µL protein sample

Protein Concentration (mg/ml)	Protein Amt (µg) in 10 µL	Absorbance @595nm		Average	Blank Reduc [Absorbance @595 nm]
	Blank	0.2938	0.2938	0.2938	
0.2	2	0.4006	0.4219	0.41125	0.11745
0.4	4	0.5121	0.5208	0.51645	0.22265
0.6	6	0.6311	0.6625	0.6468	0.353
0.8	8	0.7316	0.7646	0.7481	0.4543
1	10	0.8139	0.8349	0.8244	0.5306





SDS PAGE

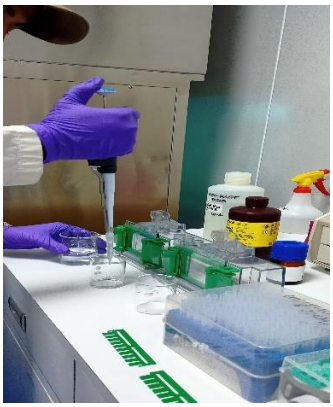
SODIUM DODICYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

SDH PAGE apparatus consist of

- Gel casting unit
- Glass plates
- Fixing clips
- Comb
- Resolving gel
- Stacking gel

RESOLVING GEL(Preparation)

- ddH2O
- 30% Acrylamide
- 0.5 M tris (PH- 8.8)
- 10% SDS
- 10% APS
- TEMED



STALKING GEL (Preparation)

- ddH2O
- 30% Acrylamide
- 0.5 M tris (PH- 6.8)
- 10% SDS
- 10% APS
- TEMED

FUNTIONS OF THE CONSTITUENTS:

1. Acrylamide: They form crosslinked gel matrix that's forms pores through which the protein passes and gets separated according to their molecular size.

Polyacrylamide gels are ideal for protein separation because it is chemically inert, electrically neutral , hydrophilic and transparent. Polyacrylamide is formed as a result of polymerization reaction between acrylamide and N,N methylene bis acrylamide using catalyst.

2. SDS : SDS is a anionic detergents that unfolds and denatured protein structure and Coats the protein with negative charge.
3. APS(Ammonium persulphate) : APS is an oxidizing agent which decomposes to form free radical.
4. TEMED(Tetramethylethylenediamine): TEMED along with APS catalyzes the polymerization of the gel matrix.

RUNNING BUFFER

- Tris base (PH 8.3)
- GLYCINE
- SDS

Running buffer contains ions that conduct current through the gel. Moreover buffer maintains the gel at a stable pH, minimizing changes that could in the protein if subjected to unstable PH.

LOADING DYE

- SDS
- GLYEROL
- Tris- HCL
- Bromophenol blue dye

IMPORTANCE OF GLYINE

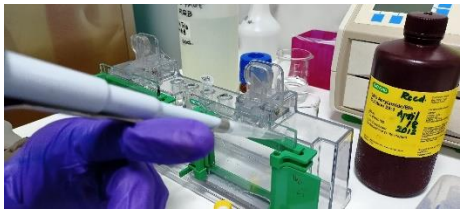
The ionic state of glycine helps to do the stacking gel do its work. Glycine is present in the running buffer having a PH of 8.3. At this PH glycine has negative charge. When an electric field is applied glycine starts moving towards the stacking gel. As soon as it enters the stacking gel at a PH of 6.8 , it becomes neutrally charged glycine zwitterions. This means that they move slowly through the gel. On the other hand, the Cl ions from Tris HCL in the gel moves at a faster rate towards the anode. When the Cl ions and glycine zwitterion hits the loading gels they create a voltage gradient in between the highly mobile Cl ions and slow moving glycine zwitterions and the proteins having electromobilities in between these both are stacked in between Cl and glycine ions. Therefore this gel is called stacking gel.

STEPS INVOLVED IN SDS- PAGE

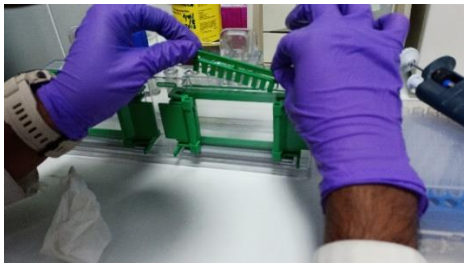
1. The gel apparatus is assembled with proper fixing of the glass plates.
2. The resolving gel is made using the required constituents.
3. The resolving gel is poured into the gel apparatus using a pipette up to a certain level.
4. 99% ethanol is then poured slowly to create a line. Ethanol helps to ensure an even interface between the two layers and also

helps to polymerize the gel faster and to come in contact of the environment.

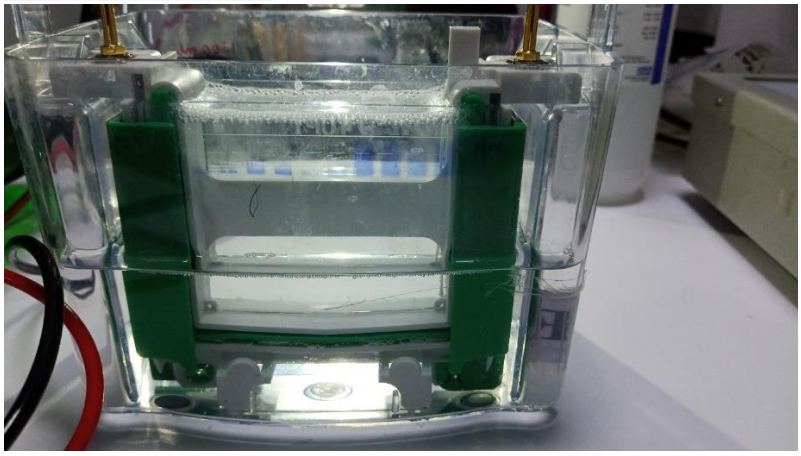
5. The stacking gel is then prepared. Ethanol is removed once the resolving gel solidifies and the slacking gel is pored.



6. Immediately combs are placed over the stacking gel to create the wells.



7. Once the gel solidifies the gels are take out and placed in a vertical electrophoresis cell. Running buffers are poured into the cell and on top of the gel. Once electric currents starts passing the proteins loaded with dye are separated.



WESTERN BLOTTING

Western blotting is a technique which is used to detect the target protein among a mixture of other proteins. It is a confirmation process which is done to detect the target protein by its transfer to a hydrophobic membrane under the influence of an electric field.

PROEDURE:

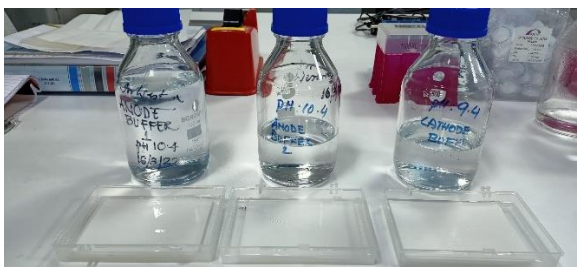
Step-1.

(Preparation of PVDF-Membrane)

- After SDS-PAGE, remove the stacking gel by cutting and discard it.
- The resolving gel is dipped in cathode buffer (pH-9.4) for 15-min.
- Take PVDF-membrane and cut according to the size of resolving gel. Keep PVDF-membrane in methanol for 15-30 sec to make it semi-transparent.
- Wash PVDF membrane using Milli-Q water for 2 min in shaker.
- Then PVDF membrane should be dipped in Anode buffer-2 (pH-10.4) for 5 min.

Step-2.

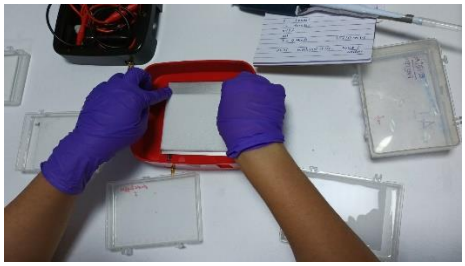
(Arrangement of Blotting paper, PVDF & Gel)



- Take 2 blotting papers and dip in Anode buffer-1 (pH-10.4) (Set-A), 1 blotting paper in Anode buffer-2 (pH-10.4) (Set-B) and 3 blotting papers in cathode buffer (pH-9.4) (Set-C).

(Note: For all dipping process should be done for 1-2 min).

- Place Set-A, on to that place Set-B, on to that place PVDF membrane, on to that place Resolving gel and on to that place Set-C.



- The entire arrangements are supplied with 15V of power for 10 min (Note: power supply is based on requirements)

Step-3.

(Antigen-Antibody(Ab) interaction).

- After completing the power supply, take PVDF membrane from arrangements and wash it with milli-Q water properly.

- Add 15ml of blocking solution, and keep it for 1-hr on shaker at RT and place at 4°C for overnight.
- Again keep it on shaker for 30-45 min at 60 rpm.
- Discard blocking buffer and wash for 3-times with TBST solution at every 10 min Interval.
- Add primary (1°) antibody (Rabbit P-ab) with the dilution ratio of 1:4000 (for 16 ml of dilution solution add 4µl of 1° Ab)
- Keep it for 1.5 h on shaker at 60 rpm
- Remove (1°) Ab solution and store in 0.02% sodium azide solution
- Wash PVDF membrane 3-times with TBST solution at every 10 min Interval
- Add secondary (2°) antibody (i.e. Goat anti-rabbit IgG) with the dilution ratio of 1:4000 (for 16 ml of dilution solution add 4µl of 2° Ab)
- Keep it for 1.5 h on shaker at 60 rpm
- Remove (2°) Ab solution and store in 0.02% sodium azide solution
- Wash PVDF membrane 3-times with TBST solution at every 10 min Interval
- Again Wash PVDF membrane with 1X-TBS solution for 1-2 min
- Add 10ml of substrate directly to membrane (Note: Substrate is photosensitive)
- After 2-3 min bands will start appearing, as bands developed, immediately remove substrate and store it
- PVDF membrane is washed with water and dried.

PROTEIN EXTRACTION FROM A MICROORGANISM AND PURIFICATION

1. **MEDIA PREPARATION**
   solid/ liquid.
2. **STREAKING OF THE MICROORGANISM**
   Culturing
3. **FERMENTATION IN THE LIQUID MEDIA.**
   Formation of broth and biomass
4. **CENTRIFUGATION**
   Seperation of broth and biomass
5. **COLLECTION OF BROTH/BIOMASS ACCORDING TO WHERE OUR PROTEIN OF INTEREST IS PRESENT**
6. **PCV (PACK CELL VOLUME)**
7. **PROTEIN ESTIMATION**
   To find the concentration of the total protein.

8. ## SDS-PAGE

To separate our target protein from a mixture of other protein with the help of markers and standard.

9. ## WESTERN BLOTTING

To confirm that the protein separated is our target protein.

10. ## CROMATOGRAPHY(PURIFICATION)

There are different chromatography process like ionic, hydrophobic, affinity etc which can be selected depending on the % of purified protein needed.

FOR AFFINITY CHROMATOGRAPHY

STEPS INVOLVED:

- Equilibration(equilibration buffer is loaded to create an equilibrium in the resin and proper environment for binding our target protein).

- Loading of the sample
- Wahing so that the other proteins get washed out.
- Elution buffer is loaded so that our target protein is eluted.
- Stripping
- Re- equilibration

11.SDS-PAGE FOR FINAL CONFIRMATION.

EPYGEN BIOTECH PRIVATE LIMITED

INTERNSHIP REPORT

DURATION: 21st July to 4th August, 2022

LOCATION: N 28/2, Addl. Patalganga

MAHARASHTRA 410222, INDIA

SUBMITTED BY:

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SUBMITTED TO:

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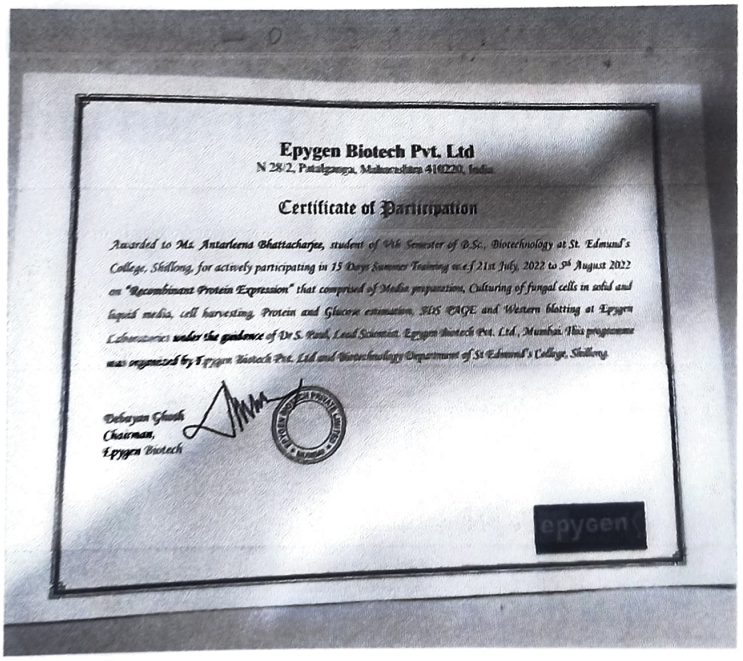

Epygen Biotech Pvt. Ltd
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Debayan Ghosh
Chairman,
Epygen Biotech

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ACKNOWLEDGEMENT

On the very onset of the internship I would like to highly express my gratitude towards Dr S. Paul , Lead scientist , Epygen Biotech pvt.Ltd ,Mumbai for allowing me to complete my internship under him . The immense knowledge and active guidance of Dr Paul helped me in gaining immense knowledge and hands on labatory experience .

INTRODUCTION

An Internship Programme was conducted from 21 st July to 5 th August in Epygen Biotech Pvt Ltd , patalganga , Mumbai . I, as a student of BSc. Biotechnology got the opportunity to be trained under this company

The main motive of this internship is "Improving the practical Skills and motivating students to pursue a career in science". It is to help the students nurture a career in different fields of Life Science. In this internship, we were taught about how Biotechnology has advanced itself throughout the years and in every field, it is necessary to have a presence of a biotechnologist. During the internship, we were taught about"RECOMBINANT PROTEIN EXPRESSION "which include making of media plates , streaking , protein estimation , SDS page , western blotting .

This training not only provided us the knowledge and the exposure to the Research field in Biotechnology but it helped us to enhance our practical skills by giving us the opportunity to do a hands-on experiment.

CONTENTS

MEDIA PREPARATION

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Materials/Reagents Needed-

1. Bovine Serum Albumin (BSA)
2. Coomassie Brilliant Blue G-250
3. Methanol
4. Phosphoric acid (H_3PO_4)
5. Bradford reagent

Equipment-

Spectrophotometer

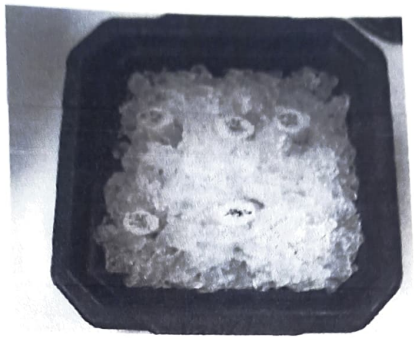
Method-

Bradford method

Procedure-

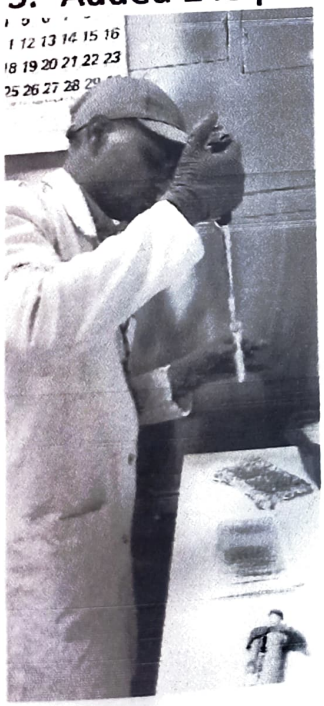
A. Standard assay procedure (for sample with 0.2-1.0 mg/ml of protein)

1. Prepared five dilutions of a protein (usually BSA) standard with a range of 0.2 mg to 1 mg protein.

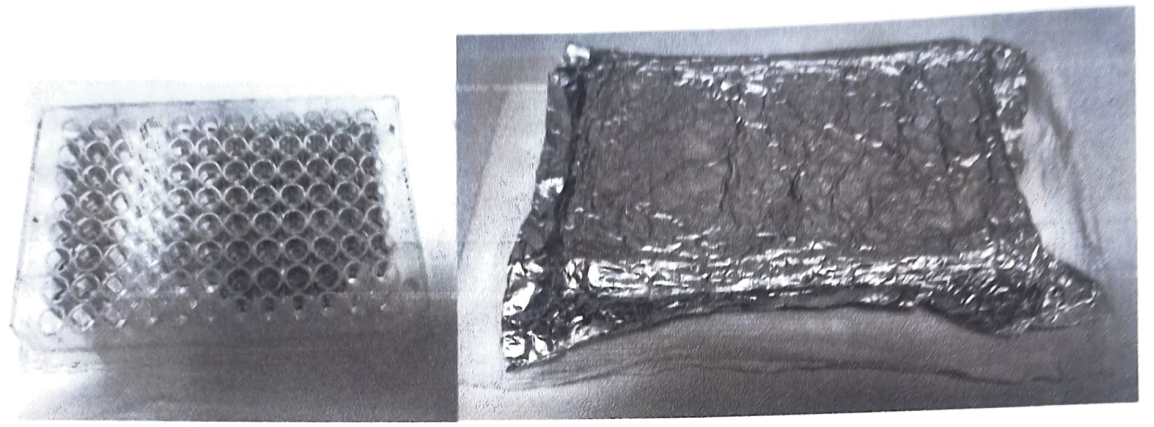


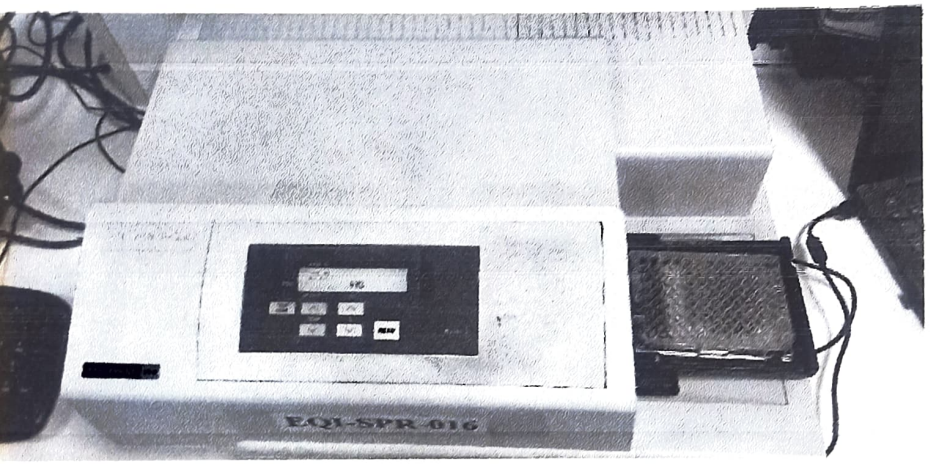
2. Diluted unknown protein samples to obtain 0.2-1.0 mg protein/ml.
3. Added 10 µl each of standard solution or unknown protein sample to an appropriately labelled test tube.
4. Set two blank tubes. For the standard curve, added 10 µl H_2O instead of the standard solution. For the unknown protein samples, added 10 µl protein preparation buffer instead. (*Note: Protein solutions are normally assayed in duplicates or triplicates*).

5. Added 240 µl of Bradford reagent to each tube and mix well.

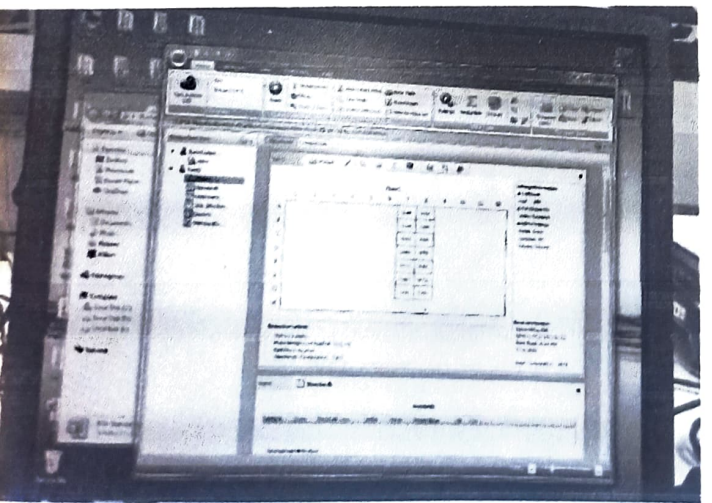


5. Incubated at room temperature (RT) for 10 min. (Note: *Absorbance increases over time; samples should be incubated at RT for no more than 15 minutes)* .
7. Measured absorbance at 595 nm





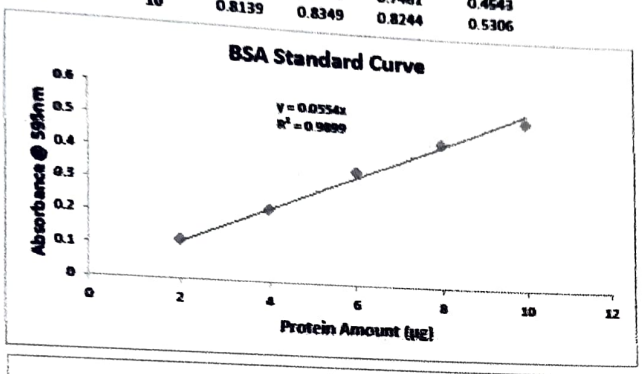
Observation and Inference-

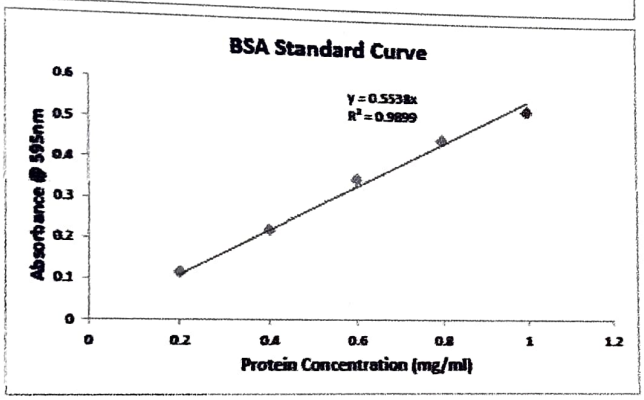


BSA STANDARDIZATION OF NEW BRADFORD REAGENT

In each well = 240 µL Bradford + 10 µL protein sample

Protein Concentration (mg/ml)	Protein Amt (µg) in 10 µl	Absorbance @595nm		Average	Blank Reduc (Absorbance @595 nm)
	Blank	0.2938	0.2938	0.2938	
0.2	2	0.4006	0.4219	0.41125	0.11745
0.4	4	0.5121	0.5208	0.51645	0.22265
0.6	6	0.6311	0.6625	0.6468	0.353
0.8	8	0.7316	0.7646	0.7481	0.4543
1	10	0.8139	0.8349	0.8244	0.5306





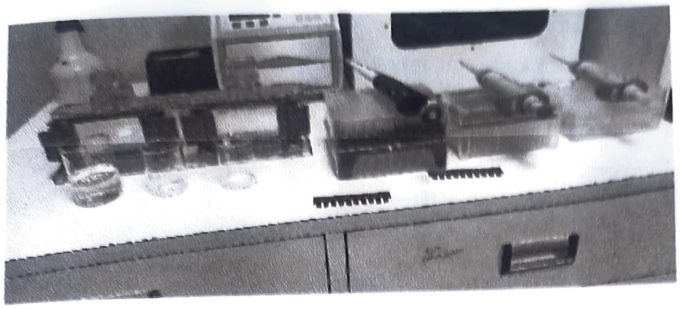
SDS PAGE

SODIUM DODICYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS

SDH PAGE apparatus consist of

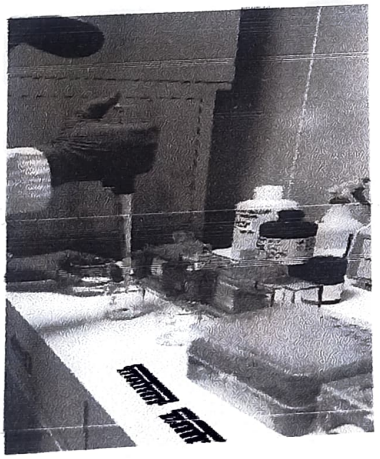
- Gel casting unit
- Glass plates
- Fixing clips
- Comb
- Resolving gel

- Stacking gel



RESOLVING GEL(Preparation)

- ddH2O
- 30% Acrylamide
- 0.5 Mtris (PH- 8.8)
- 10% SDS
- 10% APS
- TEMED



STALKING GEL (Preparation)

- ddH2O
- 30% Acrylamide
- 0.5 M tris (PH- 6.8)
- 10% SDS
- 10% APS
- TEMED

FUNTIONS OF THE CONSTITUENTS:

1. Acrylamide: They form crosslinked gel matrix that's forms pores through which the protein passes and gets separated according to their molecular size.

Polyacrylamide gels are ideal for protein separation because it is chemically inert, electrically neutral , hydrophilic and transparent. Polyacrylamide is formed as a result of polymerization reaction between acrylamide and N,N methylene bis acrylamide using catalyst.

2. SDS : SDS is a anionic detergents that unfolds and denatured protein structure and Coats the protein with negative charge.
3. APS(Ammonium persulphate) : APS is an oxidizing agent which decomposes to form free radical.
4. TEMED(Tetramethylethylenediamine): TEMED along with APS catalyzes the polymerization of the gel matrix.

RUNNING BUFFER

- Tris base (PH 8.3)
- GLYCINE

- SDS

Running buffer contains ions that conduct current through the gel. Moreover buffer maintains the gel at a stable pH, minimizing changes that could in the protein if subjected to unstable PH.

LOADING DYE

- SDS
- GLYEROL
- Tris- HCL
- Bromophenol blue dye

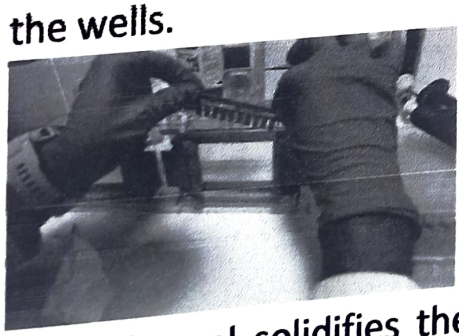
IMPORTANCE OF GLYINE

The ionic state of glycine helps to do the stacking gel do its work. Glycine is present in the running buffer having a PH of 8.3. At this PH glycine has negative charge. When an electric field is applied glycine starts moving towards the stacking gel. As soon as it enters the stacking gel at a PH of 6.8 , it becomes neutrally charged glycine zwitterions. This means that they move slowly through the gel. On the other hand, the Cl ions from Tris HCL in the gel moves at a faster rate towards the anode. When the Cl ions and glycine zwitterion hits the loading gels they create a voltage gradient in between the highly mobile Cl ions and slow moving glycine zwitterions and the proteins having electromobilities in between these both are stacked in between Cl and glycine ions. Therefore this gel is called stacking gel.

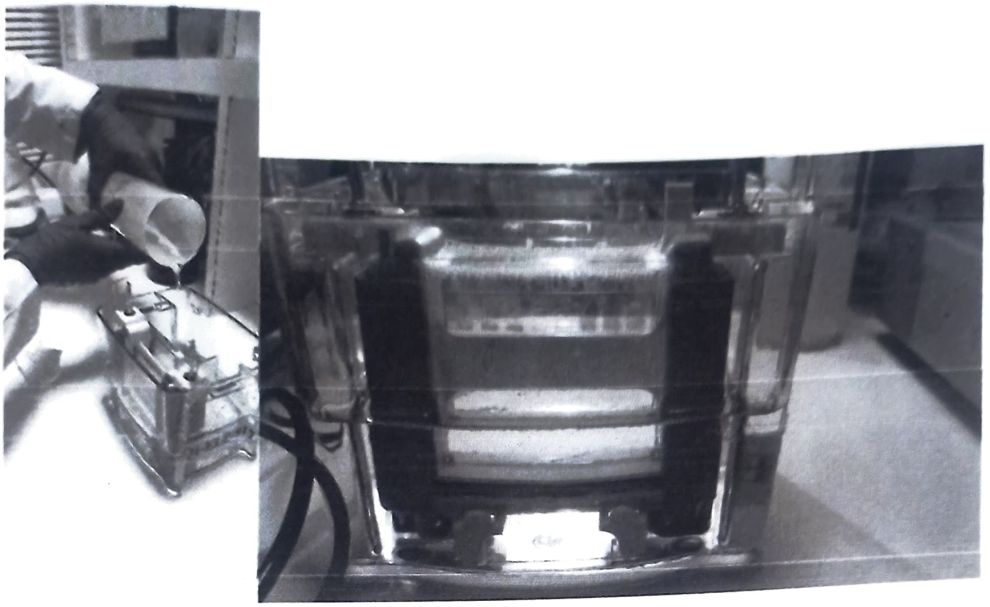
STEPS INVOLVED IN SDS- PAGE

1. The gel apparatus is assembled with proper fixing of the glass plates.
2. The resolving gel is made using the required constituents.
3. The resolving gel is poured into the gel apparatus using a pipette up to a certain level.
4. 99% ethanol is then poured slowly to create a line. Ethanol helps to ensure an even interface between the two layers and also helps to polymerize the gel faster and to come in contact of the environment.
5. The stacking gel is then prepared. Ethanol is removed once the resolving gel solidifies and the slacking gel is pored.

6. Immediately combs are placed over the stacking gel to create the wells.



7. Once the gel solidifies the gels are take out and placed in a vertical electrophoresis cell. Running buffers are poured into the cell and on top of the gel. Once electric currents starts passing the proteins loaded with dye are separated.



Western blotting is a technique which is used to detect the target protein among a mixture of other proteins. It is a confirmation process which is done to detect the target protein by its transfer to a hydrophobic membrane under the influence of an electric field.

PROEDURE:

Step-1.

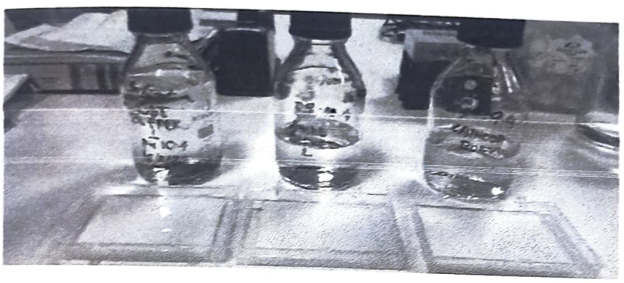
(Preparation of PVDF-Membrane)

- After SDS-PAGE, remove the stacking gel by cutting and discard it.
- The resolving gel is dipped in cathode buffer (pH-9.4) for 15-min.

- Take PVDF-membrane and cut according to the size of resolving gel. Keep PVDF-membrane in methanol for 15-30 sec to make it semi-transparent.
- Wash PVDF membrane using Milli-Q water for 2 min in shaker.
- Then PVDF membrane should be dipped in Anode buffer-2 (pH-10.4) for 5 min.

Step-2.

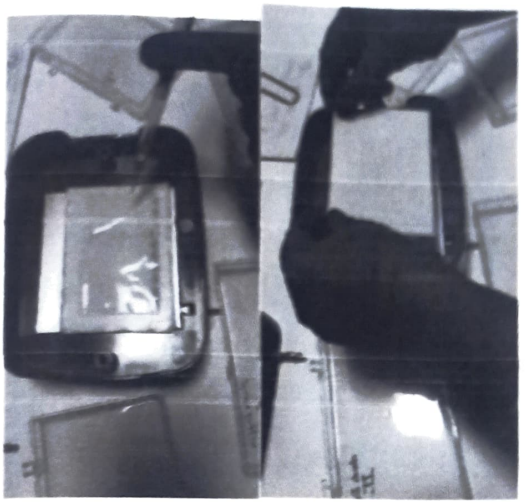
(Arrangement of Blotting paper, PVDF & Gel)



- Take 2 blotting papers and dip in Anode buffer-1 (pH-10.4) (Set-A), 1 blotting paper in Anode buffer-2 (pH-10.4) (Set-B) and 3 blotting papers in cathode buffer (pH-9.4) (Set-C).

(Note: For all dipping process should be done for 1-2 min).

- Place Set-A, on to that place Set-B, on to that place PVDF membrane, on to that place Resolving gel and on to that place Set-C.



- The entire arrangements are supplied with 15V of power for 10 min (*Note: power supply is based on requirements*)

Step-3.

(Antigen-Antibody(Ab) interaction).

- After completing the power supply, take PVDF membrane from arrangements and wash it with milli-Q water properly.
- Add 15ml of blocking solution, and keep it for 1-hr on shaker at RT and place at 4°C for overnight.
- Again keep it on shaker for 30-45 min at 60 rpm.
- Discard blocking buffer and wash for 3-times with TBST solution at every 10 min Interval.
- Add primary (1°) antibody (Rabbit P-ab) with the dilution ratio of 1:4000 (for 16 ml of dilution solution add 4µl of 1° Ab)
- Keep it for 1.5 h on shaker at 60 rpm
- Remove (1°) Ab solution and store in 0.02% sodium azide solution
- Wash PVDF membrane 3-times with TBST solution at every 10 min Interval

- Add secondary (2°) antibody (i.e. Goat anti-rabbit IgG) with the dilution ratio of 1:4000 (for 16 ml of dilution solution add 4µl of 2° Ab)
- Keep it for 1.5 h on shaker at 60 rpm
- Remove (2°) Ab solution and store in 0.02% sodium azide solution
- Wash PVDF membrane 3-times with TBST solution at every 10 min Interval
- Again Wash PVDF membrane with 1X-TBS solution for 1-2 min
- Add 10ml of substrate directly to membrane (Note: Substrate is photosensitive)
- After 2-3 min bands will start appearing, as bands developed, immediately remove substrate and store it
- PVDF membrane is washed with water and dried.

PROTEIN EXTRACTION FROM A MICROORGANISM AND PURIFICATION

1. **MEDIA PREPARATION**
   solid/ liquid.
2. **STREAKING OF THE MICROORGANISM**

Culturing

3. **FERMENTATION IN THE LIQUID MEDIA.**
   Formation of broth and biomass
4. **CENTRIFUGATION**
   Seperation of broth and biomass
5. **COLLECTION OF BROTH/BIOMASS ACCORDING TO WHERE OUR PROTEIN OF INTEREST IS PRESENT**
6. **PCV (PACK CELL VOLUME)**
7. **PROTEIN ESTIMATION**
   To find the concentration of the total protein.

8. **SDS-PAGE**
   To separate our target protein from a mixture of other protein with the help of markers and standard.
9. **WESTERN BLOTTING**
   To confirm that the protein separated is our target protein.

10. CROMATOGRAPHY(PURIFICATION)

There are different chromatography process like ionic, hydrophobic, affinity etc which can be selected depending on the % of purified protein needed.

FOR AFFINITY CHROMATOGRAPHY

STEPS INVOLVED:

- Equilibration(equilibration buffer is loaded to create an equilibrium in the resin and proper environment for binding our target protein).
- Loading of the sample
- Wahing so that the other proteins get washed out.
- Elution buffer is loaded so that our target protein is eluted.
- Stripping
- Re- equilibration

11. SDS-PAGE FOR FINAL CONFIRMATION.

A Summer Training report
On
Basic Microbiology

Organized by

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JULY' 22

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I sincerely express my gratitude towards the St.Edmund's College for providing this opportunity.

Secondly I would also like to thank my parents and friends who helped me a lot in finalizing this report within the limited time frame.

Date : 8.08.22

Place :Shillong

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Dept. of Biotechnology-III Yr.

Vels Institute of Science, Technology and Advanced Studies.

INTRODUCTION

The **Summer Training Programme** at St. Edmunds College, **Biotechnology Department** Designed to provide the biotech interns with knowledge about the fundamentals of laboratory procedures, from a thorough run-through of all the tools in the lab to develop the research approach for a project that has been completed.

The majority of the curriculum was devoted to provide the interns with practical experience in isolating bacterial colonies from a specific source and identifying the bugs using various staining technique and biochemical testing. The same programme contained **isolation of genomic DNA** and plasmid from a particular bacteria and running **agarose gel electrophoresis** to determine the species. The application also provided accurate calculation of chemical reagent for the preparation of various medium required for the isolation procedures. The final phase of the curriculum comprised classes on **research methodology and biostatistics,** where the fundamentals and significance of biostatistics are imparted to us and the proper framework for developing a research methodology was taught.

TABLE OF CONTENTS

GOOD LABORATORY PRACTICES

One of the fundamental purposes of the Principles of Good Laboratory Practice (GLP) is to ensure the quality and integrity of test data related to non-clinical safety studies. The way in which study data, supporting human, animal and environmental safety assessment, is generated, handled, reported, retained and archived has continued to evolve in line with the introduction and ongoing development of supporting technologies. However, the main purpose of the requirements of the Principles of GLP remains the same in having confidence in the quality, the integrity of the data and being able to reconstruct activities performed during the conduct of non-clinical safety studies.

There are some rules which must be observed for the successful completion of the laboratory exercise, personal safety and convenience of others working in the laboratory:

- Attending all required laboratory safety training prior to the start of the research assignment.
- Reading all procedures and associated safety information prior to the start of an experiment.
- Performing only those experiments authorized by the supervisor.
- To follow all written and verbal instructions. Ask for assistance if any guidance is required.
- Working under direct supervision at all times.
- The locations and operating procedures for all safety equipments should be known. This includes the eyewash station and safety shower.
- Knowing the locations of the nearest fire alarms and at least two ways out of the building. Never to use an elevator in emergencies.
- Being alert and proceeding with caution at all times in the laboratory. Immediately notify the supervisor of any unsafe conditions.
- Knowing the proper emergency response procedures for accidents or injuries in the laboratory.
- Practicing good personal hygiene. Washing hands after removing gloves, before leaving the laboratory, and after handling a potentially hazardous material.
- While working in the laboratory, we should wear personal protective equipment - eye protection, gloves, and laboratory coat - as directed by the supervisor.
- Proper segregation and disposal of all laboratory waste.
- Dressing for work in the laboratory. Wearing clothing and shoes that cover exposed skin and protect from potential splashes. Tieing back long hair, jewelry, or anything that may catch in equipment.
- Never to eat food, drink beverages, chew gum, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
- Usage of a chemical fume hood or biosafety cabinet, as directed by the supervisor.
- Observing good housekeeping - keeping aisles clear.
- Report damaged electrical equipment to the supervisor. Do not use damaged electrical equipment.

DEMONSTRATION OF ALL INSTRUMENTS

Microbiology is a specialized branch of biological sciences. Microorganisms are all around us and play a special role in the ecology of life on earth. According to Louis Pasteur “Life would not long remain possible in the absence of microbes”. However, some microorganisms cause diseases in humans, other animals and plants. There is, as in all sciences, a need for basic equipment, much of which can be found in any biological laboratory. Following is a list of basic requirements which a microbiologist requires in his laboratory for microscopic examination, isolation or culturing and identification of a microorganism as well as to study its structure, function and application.

The most commonly used equipment is inoculation needles, transfer loops, inoculation, Bunsen burner, autoclave (or pressure cooker) incubators, hot air oven centrifuge, spectrophotometer magnetic stirrer electric shaker and rotary shaker heating plate, heating mantle distillation plant, UV-lamp carbon dioxide cylinder, water-bath and a single-pan balance that has weights (for general use) chemical balance, fine analytical balance pH meters, Quebec colony counter, Laminar air flow, camera lucida electrophoresis and a high-quality microscope and many more.

Here are some of the instruments that has been used in the summer training:

1. Inoculation needle and inoculation loop

- They are among the most frequently employed tools.
- The Inoculation needle/loop consists of a platinum wire that is welded to a metallic rod.
- Wire loops have a handle that is fitted with a steel screw shaft. Metallic rod nichrome, or platinum wire will be put into.
- The wire is be wrapped around a small circular object like a pencil or other similar objects. To create a loop making it twist mechanically. The loop must be designed as to keep an elongated film within it, by dips in the solution. In order to do this, a dimension (5-7 millimeters) that the wire has is suggested.
- Straight wire or straight needle is made of wire instead of loop. The free or open part of it is sharp. Straight and loop wire must be sterilized through either Bunsen burners or the hot heating coil until the loop or needle turn hot red. When the loop or wire has cooling, they are typically utilized for transferring cultures out of liquid broth.

- The straight needle is utilized to transfer the culture out of solid media. A smaller quantity of liquid culture may be controlled using a straight needle.
- The loop and wire can be are used to extract small amounts of solid material from a microbial colony and also to inoculate liquid or solid medium. Both the straight and loop wire need to be heated right after use, so that contamination is prevented.

2. Waterbath

- Waterbath is an instrument which is utilized to supply an unchanging temperature to the sample
- It's a small insulating box made of steel, and fitted with an electrode of an electric heating coil.
- It is also controlled via the thermostat.

3. Autoclave

The killing effects of heat on living organisms can be achieved through the increase of steam pressure in an enclosed system. The water molecules are consolidated which results in an increase in their permeation. The water is boiling at 100 degrees and steam builds up in a closed vessel, leading to an increased pressure. This relationship between temperature and pressure is illustrated below.

- The autoclave is generally composed of pressure cookers composed of gun-metal sheets, which are held in the aluminum case.
- It's closed with a swing doors that are secured to the wall by bolts with a radical design.
- In laboratories for microbiology, an autoclave with a horizontal system that is jacketed is essential.
- The steam flows from below at the bottom. The walls on the sides will be heated through the jacket. It is equipped to keep track of the pressure.
- There is a way to regulate the pressure with a pressure meter. It is a security valves that protect against any accidents. It's based on moist heat, which is can be used for sterilisation.
- The Materials required for the experiment such as the glassware, petri plates, conical flask, haemocytometre, etc. are nicely covered and autoclave to kill all the contaminants present in the equipments.

4. Laminar Air Flow

Laminar flow is an instrument that is comprised of an air compressor located in the rear of the chamber, which is able to create air flow at the same velocity across parallel lines of flow. It has a specific filtering system that is high-efficiency particles in the air **(HEPA**) that is able to remove particles that are as small **as 0.3 millimeters.**

- In front of the blower there is a mechanism which the air that is blown out of the blower creates air velocity along flow lines that are parallel.
- The concept of laminar flow relies on the flow of an air current that is uniform in velocity through parallel flow lines, which assist in the transfer of microbial culture under an aseptic condition. Air flows through filters and then into the enclosure. The filters do not permit any type of microbes to enter the system.
- Within the chamber, one fluorescent tube and another UV tube are installed. Two switches for these tubes as well as an additional switch to regulate of airflow are installed on the device. Because of the uniform speed and the parallel flow of air current the pouring of media, plating. Slant preparations, streaking etc. Without any contamination are carried out.
- At first dust particles are removed off the surfaces of flow by using a smooth cloth that is infused with alcohol. Turn on **the UV light** for a time of 30 minutes in order to eliminate germs that are found in the work space.
- Front cover of device is opened to keep the material which is to be kept within. It is set to the appropriate level so that the air in the chamber is removed as the air in the chamber could be contaminated or carry contaminants.
- All work related to pouring or plating, streaking etc. must be completed within the flame area of the burner or lamp.

5. Incubator

- An incubator is a device comprised of a steel/copper chambers, in which air or warm water circulates by electrical current or through a small gas flames.
- Incubator temperature is maintained steady due to its control via a thermostat.
- The incubator is made of a double walled chamber that can be that can be adjusted to the desired temperature. This is accomplished by an external knob to control temperature control. The space between walls then insulated to test the heat conduction. A thermometer is placed from the top to record the temperature. Nowadays, sophisticated incubators are made available with oxygen and humidity control systems.

- The temperature has a significant impact on development of microbial. So, instruments are typically constructed to permit the microorganism that is wished to thrive at a specific temperature.
- It's designed to allow the growth of microbial colonies in the appropriate medium at the right temperature. When using an incubator, difference in temperature shouldn't exceed one degree.

7. Electrical balance

- It works with the help of electricity and displays the digital weights display.
- It is comprised of a single pan that weighs a single pan. Its weight is counterbalanced with weights, and is set to zero.
- The weighted material is put on the balance pan and the counterweights required are removed using the knobs. As time passes, the digital scale starts moving between up and down.
- Always take off the counter-weights that are proportional to the material's weight.

8. Spectrophotometer (Colorimeter)

- Spectrophotometer is a tool which makes use of the light source as an instrument for radiation. It also measures changes in optical density, or absorbance.
- It is based on three fundamental principles: (i) the radiation source, (ii) a unit to disperse radiation across various wavelengths and (iii) an instrument that can will detect how much radiation is present that is detected at different wavelengths.
- Spectrophotomete employs the monochromatic (narrow frequencies) radiation, while colorimeter employs wide wavelengths.
- There are a variety of atoms as well as their electron clouds within every chromophore (a chemical molecule or a portion that is). Because of changes in the energy levels of electrons, the form of the chromophore can be altered. These variations in energy are recorded using any spectrophotometer.

9. Centrifuges

The centrifuge is a device which rotates at a high rate and separates particles or substances by the density and mass with the help by centrifugal force. The force exerted by the centrifuge is measured in terms of revolutions every minutes (rpm) of the angular speed. A centrifuge is comprised of the "head" which is rapidly rotated with an up-right motor. Usually, four containers or cups are connected to the head to hold the tubes and other vessels made of the material that the particulate matter will be separated. When

centrifugation is performed, the liquid that contains particles is kept inside tubes and moves at a specific speed, and, when the centrifugation process is completed the particles are settled on lower levels of tubes. The various types of centrifuges typically used include slow-speed (clinical centrifuge) and high-speed, also known as ultracentrifuge and superspeed. The maximum speed limits of ultracentrifuges with low-speed, high-speed or low-speed speeds are **5500 rpm, 18000 rpm** and **20000-60000 rpm** and **20000 to 60000 rpm,** respectively. They are utilized for the separation of particles in suspended matter removal of liquid mixtures with varying in density and solids or liquids the concentration of microorganisms within various samples for studies of enzymatic activity.

10. Microscope

For the study of basic and applied microbiology. The microscope is an essential tool in all the tools needed in the laboratory. The microorganisms in tiny sizes are visible only through a microscope and are inaccessible to naked eye. Some of them are colorless, but the microscope with the phase contrast attachment can see them with high resolution. Antoni van Leeuwenhoek (1673) invented in the field of science biconcave lenses that were positioned between two plates made of steel that had a magnification of **300X** as a microscope.

The microscope was utilized by him to observe microbes. The electron microscope today is capable of magnifying over **250,000X** of the specimen. There are two main kinds of microscopes are offered that include light microscopes, which comprise dark field, bright field as well as phase contrast and electron microscopes and fluorescent microscopes, its function is based on the electron beam and magnets to view submicroscopic images. The most basic microscope is comprised of an eyes **(10X magnification)** which is inserted inside the tube of the microscope.

CHEMICAL CALCULATION AND REAGENT/MEDIA PREPARATION

Requirements:

1. Peptone
2. Beef Extract
3. NaCl
4. Distilled water
5. Agar powder

Procedure

- So, at first 400ml of distilled water is taken into a conical flask of 1000ml. To which measured amount of peptone, beef extract, NaCl and Agar are to be taken.
- Nutrient Broth and Agar*
- The amount of **peptone** taken in 1000ml of distilled water is 5g
- Therefore, in 1ml water the amount of **peptone** taken is = 5/1000g
- So, the amount of **peptone** taken in 400ml of water = 5/1000g×4000 = 2g
- The amount of **Beef exatract** taken in 1000ml of distilled water is 3g
- Therefore, in 1ml water the amount of **Beef extract** taken is = 3/1000g
- So, the amount of **Beef extract** taken in 400ml of water = 3/1000g×4000 = 1.2g
- The amount of **NaCl** taken in 1000ml of distilled water is 5g
- Therefore, in 1ml water the amount of **NaCl** taken is = 5/1000g
- So, the amount of **NaCl** taken in 400ml of water = 5/1000g×4000 = 2g
- The amount of **Agar** taken in 1000ml of distilled water is 15g
- Therefore, in 1ml water the amount of **Agar** taken is = 15/1000g

- So, the amount of **Agar** taken in 400ml of water = 15/1000g×4000 = 6g

- *For nutrient agar we have added 6g agar. **Mainly used for isolation of bacteria and actinomycetes.**

- After measuring the amount of the ingridients, these are poured into the conical flask filled with 400ml of water. The solution is mixed and then heated into the microwave for several minutes at high temperature until dissolved completely. A broth is formed.

Culture media is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible, and repeatable microbiological test results is reduced. A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms. Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents.

ISOLATION OF MICROORGANISM

Requirements

- Sample (soyong squash) in a sterile bottle
- Sterile Petri-plates
- Micropipette
- Nutrient Agar media
- Test tubes

Procedure

- The Petri-plates, the culture media and Micropipette is taken into the Laminar Flow (We should first Make sure that the Laminar Flow is Cleaned properly with **70% Ethanol** And **UV light** is switched on and kept for 15 mins to kill any kinds of microbes present in it).
- The nutrient culture media is poured into the Petri-plates covering 2/3rd of it. We have to make sure that the plates are covered with lid instantly after pouring the media so that it is not attacked by microbes.
- After pouring, the plates are to be kept inside the laminar flow until it solidifies. In the mean time, the serial dilution method is applied. After the dilution, small amount of the sample as well as diluted media is taken in each plates and then inoculated. Bacterial culture growth is observed.

Culture media has been used by microbiologists since the nineteenth century. Even with the increased use of rapid methods the majority of techniques found in the pharmaceutical quality control laboratory require growth media. For the assessment of culture media, no one definitive standard exists.

Culture media is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible, and repeatable microbiological test results is reduced. A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms. Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents.

Since there are many types of microorganisms, each having unique properties and requiring specific nutrients for growth, there are many types based on what nutrients they contain and what function they play in the growth of microorganisms.

SERIAL DILUTION

Serial dilution is a laboratory technique, in which a stepwise dilution process is performed on a solution with an associated dilution factor. In the laboratory, this method is used to decrease the counts of viable cells within a culture to simplify the operation.

In serial dilution, the cell count or density gradually decreases as the serial number increases in each step. This makes it easier to calculate the cell numbers in the primary solution by calculating the total dilution over the whole series.

The following is the serial dilution procedure for a ten-fold dilution of a sample to a dilution factor of 10-6:

- 10 sterile and clean test tubes are taken.
- The selected sample Sohiong squash (Prunus nepalensis) is taken into a test tube and the remaining test tubes are filled with 9 ml of distilled water.
- 1ml of sample into the sterile pipette is drawn. The sample must be properly mixed, if necessary use a vortex meter.
- Then this 1ml sample is transferred within the first test tube to make the total volume of 10 ml. It provides an initial dilution of 10-1. It should be made sure during the transfer, the tip of pipette doesn't touch the wall of test tube or no amount of sample remains at the tube wall.

In serial dilution technique, the dilution factor can be calculated either for a single test tube or for the entire series (total dilution factor).

In the case of ten-fold dilution, where 1ml of sample is transferred to 9 ml of diluent, the dilution factor for that test tube will be:

Dilution factor = 1ml/1ml + 9ml = 1/10

The samples and the diluted samples are marked as S1, S2……..S9. Then with the help of a pipette 1ml of the sample and the diluted sample is taken and spread in the solidified nutrient media. After this the plates are placed inside the incubator for incubation.

PURIFICATION OF MICROORGANISMS

Streak-plate method

The streak-plate method offers a most practical method of obtaining discrete colonies and pure cultures. It was originally developed by two bacteriologists, **Leoeffler** and **Graffkey** in the laboratory of Robert Koch. In this method, a sterilized loop is dipped into a suitable diluted suspension of organisms which is then streaked on the surface of an already solidified agar plate to make a series of parallel, non-overlapping streaks.

Requirements

- Isolated sample
- Nutrient agar plates
- Inoculating loop
- Bunsen burner.

Procedure

- The inoculating loop was sterilized in the Bunsen burner by putting the loop into the flame until is it red hot.
- An isolated colony is picked from the agar plate culture and was spread over the first quadrant.
- Immediately the inoculating loop is streaked very gently over a quarter of the plate using a back and forth motion.
- The loop is flamed again and allowed to cool. Going back to the edge of area 1 that was just streaked, the streaks were extended into the second quarter of the plate.
- The loop is flamed and allowed to cool. Going back to the area that was just streaked, the streaks were extended into the third quarter of the plate.
- The loop is flamed again and allowed it to cool. Going back to the area that was just streaked, the streaks were extended into the center fourth of the plate.
- The loop is flamed once more.

STAINING DAY TECHNIQUES

Gram staining of bacteria

The Gram stain, a differential method was developed by Dr. Hans Christian Gram, a Danish physician, in1884 that is why Gram staining. In this process, the fixed bacterial smear is subjected to four different reagents in the order listed: crystal violet, iodine solution, alcohol and safranin. The bacteria which retain the primary stain (appear dark blue or violet) (i.e. not decolorized when stained with Gram's Method) are called gram-positive, whereas those that lose the crystal violet and counter stained by safranin (appear red) are referred to as gram-negative.

Requirements

- Gram staining reagents:
    - Crystal violet
    - Gram's iodine solution
    - 95 percent ethyl alcohol
    - Safranin
- Staining tray
- Wash bottle of distilled water
- Droppers
- Inoculating loop
- Glass slides
- Blotting paper
- Lens paper
- Bunsen burner
- Microscope

Procedure

- Thin smears of isolated sample was made on separate glass slides.
- The smears was air dried.
- The smears was held using slide rack.
- The slides was covered with crystal violet for 30 seconds.
- Each slide was washed with distilled water for a few seconds, using wash bottle.
- Each smear was covered with Gram's iodine solution for 60 seconds.
- The iodine solution is washed off with 95 percent ethyl alcohol. Ethyl alcohol was added drop by drop, until no more colour flows from the smear.
- The slides was washed with distilled water and drained.

- Saffranin is added to smears for 30 seconds.
- It was then washed with distilled water and blot dry with absorbent paper.
- The stained slides were allowed to air dry.

Gram Negative Bacterial Preparation

- Harvesting of cells
    - 1.5ml of an overnight bacterial broth culture were pelleted by centrifuging for 2 minutes at 12,000-16,000 g. The culture medium were removed and discarded.
- 2. Resuspend cells
    - The pellet were resuspended thoroughly in 180 microlitre of Lysis Solution.
- Prepare for cell lysis
    - 20 microlitre of the Proteinase K solution were added to the sample. It was then mixed and incubated for 30 minutes at 55degree Celsius.
- Lysis cells
    - 20 microlitre of lysis Solution, were added, vortex thoroughly and incubated at 55 degree Celsius for 10 minutes.

Capsule staining

The main purpose of capsule stain is to distinguish capsular material from the bacterial cell. A **capsule** is a gelatinous outer layer secreted by bacterial cell and that surrounds and adheres to the cell wall. Most capsules are composed of polysaccharides, but some are composed of polypeptides. The **capsule** differs from the **slime layer** that most bacterial cells produce in that it is a thick, detectable, discrete layer outside the cell wall. The capsule stain employs an acidic stain and a basic stain to detect capsule production.

Requirements

- Isolated sample
- **Crystal Violet (1%)**
  Crystal Violet (85% dye content) = 1 gm.
  Distilled Water = 100 ml
- **Nigrosin**
  Nigrosin, water soluble = 10 gm.
  Distilled Water = 100 ml
- Staining tray

- Glass slides
- Inoculating loop
- Blotting paper
- Spirit lamp
- Microscope.

Procedure

- A small drop of a negative stain is placed (India Ink, Congo Red, Nigrosin, or Eosin) on the slide.
  Congo Red *is easier to see, but it does not work well with some strains.* ***India Ink*** *generally works, but it has tiny particles that display Brownian motion that must be differentiated from your bacteria.* ***Nigrosin*** *may need to be kept very thin or diluted.*
- Using sterile technique, a loopful of bacterial culture is added to the slide, smearing it in the dye.
- Using the other slide, the ink-cell mixture is dragged into a thin film along the first slide and allowed to stand for **5-7 minutes.**
- Allowed to air dry (do not heat fix).
- The smear is then flooded with **crystal violet stain** (this will stain the cells but not the capsules) for about **1 minutes**. The crystal violet is drained by tilting the slide at a 45 degree angle and letting the stain run off until it air dries.
- The smear is examined microscopically (40X) for the presence of encapsulated cells as indicated by clear zones surrounding the cells.

BIOCHEMICAL TESTS

Catalase Test

This test demonstrate the presence of catalase, an enzyme that catalysis the release of oxygen from hydrogen peroxide.

Procedure

- A loop was used to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
- A drop of 3% hydrogen was placed in a glass slide.
- The evolution of oxygen bubbles was observed.

Oxidase Test

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme.

Procedure

- Strip of Whatman's No. 1 filter paper was soaked in 1% solution of tetramethyl-p-pheneylene-diaminedihydrochloride and let it dry.
- A sterile loop was used to pick a well-isolated colony from a fresh bacterial plate and rub it onto treated filter paper.
- Color changes was observed.

Glucose fermentation test

Oxidative/fermentation glucose test (OF glucose test) is a biological technique that was developed in 1953 by Hugh and Leifson to be utilized in microbiology to determine the way a microorganism metabolizes a carbohydrate such as glucose (dextrose).

Procedure

- Allow medium to warm to room temperature prior to inoculation.
- Inoculate the Purple Broth (with carbohydrate of choice) with isolated colonies from an 18-24 hour pure culture of the organism.
- Inoculate a control tube of Purple Broth Base in parallel with the carbohydrate based media.
- Incubate inoculated media aerobically at 35-37ºC. For 3-5 days.
  Note: Increased incubation up to 30 days may be necessary for some microorganisms.

- Observe daily for development of a yellow colour in the medium.

ISOLATION OF GENOMIC DNA FROM BACTERIA

Principle

Isolation of genomic DNA is one of the most important and common experiment that is carried out in molecular biology and includes the transition from cell biology to molecular biology. The most common method of isolating genomic DNA without the use of commercial kit is by phenol/chloroform method. So, the basic objective of this test is to study the method of isolation genomic DNA from E.Coli.

Reagents and chemicals*:*

- Tris base
- Proteinase K
- Phenol/chloroform(1:1)
- 200 proof ethanol
- RNAse
- Ethanol
- SDS
- EDTA
- Tryptone
- Yeast Extract
- NaCl
- LB Medium (1%tryptone, 0.5% yeast extract, 200 mM NaCl)
- TE buffer (10mM Tris-Cl (pH 8.0), I mM EDTA (pH 8.0))

Equipment

- Lysis buffer(10ml)(9.34ml TE buffer, 600 ul of 10% SDS, 60μl of Proteinase K(20 mg/ml))
- Tabletop centrifuge (Eppendorf)
- 1.5ml Eppendorf tube
- Incubator & Gloves

Procedure

- At first 1.5ml of overnight E.coli culture that was grown in LB medium is taken and transferred into a 1.5ml of Eppendorf tube and then centrifuged at maximum possible speed for about 1 min to extract the cell pellet.
- The supernatant is then discarded and the pellet is resuspended in 600 microliter of lysis buffer and completely vortexed to mix it properly.
- It is incubated for 1 hour at 37ºC. Equal volume of phenol/chloroform is added and mixed properly.
- It is then spun at maximum speed for about 5 min which leads to the formation of a white layer in the aqueous phenol/chloroform interface.
- The aqueous phase is then transferred to a new tube very carefully through 1ml pipette. The above two steps can be repeated till the white layer disappears. To remove the phenol equal volume of chloroform is added to the aqueous layer.
- The mixture is mixed properly and spun at maximum speed for about 5min. Then the aqueous layer is transferred to a new tube.
- For precipitation of the DNA, 2.5ml of cold ethanol is added and mixed properly. Precipitation may cause diffusion. The tube requires to be kept at -20ºC for about 30min and then spun. This way one will be able to see the DNA pellet.
- It then spun for about 15min at 4ºC. The supernatant is discarded and the DNA pellet is rinsed with 1ml of 70% ethanol.
- Then again it is spun at maximum speed for about 2 min and the supernatant is discarded while the DNA pellet is washed with 1ml of 70% ethanol.
- Now it's again spun at maximum speed.
- At last the DNA is resuspended in TE buffer.

ISOLATION OF PLASMID DNA

Principle

In prokaryotes, plasmid is double stranded, circular, and is found in the cytoplasm. The cell membranes must be disrupted in order to release the plasmid in the extraction buffer. Solution 1 contains glucose, Tris, and EDTA. Glucose provides osmotic shock leading to the disruption of cell membrane, Tris is a buffering agent used to maintain a constant pH8. Plasmid can be protected from endogenous nucleases by chelating Mg2++ ions using EDTA. Mg2++ ion is considered as a necessary cofactor for most nucleases. Solution II contains NaOH and SDS and this alkaline solution is used to disrupt the cell membrane and NaOH also denatures the DNA into single strands. Solution III contains acetic acid to neutralise the pH and potassium acetate to precipitate the chromosomal DNA, proteins, along with the cellular debris. Phenol /chloroform is used to denature and separate proteins from plasmid. Chloroform is also a protein denaturant, which stabilizes the rather unstable boundary between an aqueous phase and pure phenol layer. The denatured proteins form a layer at the interface between the aqueous and the organic phases which are removed by centrifugation. Once the plasmid DNA is released, it must be precipitated in alcohol. The plasmid DNA in the aqueous phase is precipitated with cold (0o C) ethanol or isopropanol. The precipitate is usually redissolved in buffer and treated with phenol or organic solvent to remove the last traces of protein, followed by reprecipitation with cold ethanol.

Materials Required

Luria Broth

Bacterial cells containing plasmid

Reagents TE buffer (pH 8.0)

Solution I

Solution II

Solution III

Phenol-chloroform mixture

Isopropanol

70% ethanol

1% agarose gel and an electrophoresis apparatus

Autoclaved Distilled Water

Eppendorf tubes 2 ml

Micropipette

Microtips

Microfuge

Preparation of Reagents:

1. TE BUFFER (pH 8.0): 10 mm Tris HCl (pH 8.0) 1 mm EDTA (pH 8.0)

2. Solution I: Lysis solution: 50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 mg/mL RNAse A, (store at 4˚C)

3. Solution II: Denaturing solution: 0.2 M NaOH; 1 % (w/v) SDS

4**. Solution III: Neutralizing solution**: 3 M Potassium acetate, pH 5.5

5. **PHENOL – CHLOROFORM MIXTURE**: Mix equal volume of phenol with chloroform. Keep the mixture on ice and add 20 ml TE buffer, extract by shaking for 15 minutes. Remove the dust on the surface layer using a pipette. Repeat 4-5 times. Add 30-40 ml of TE buffer and store it in dark.

6. Isopropanol

PROCEDURE:

- 2ml of overnight culture is taken and centrifuged for 5 minutes. The supernatant is then discarded carefully.
- 100µl of solution I is added to the cell pellet and resuspended by mixing gently.
- The above mixture is incubated at room temperature for 5 minutes.
- Thereafter 200µl of solution II is added to the mixture and mixed by inverting the tube.
- The above mixture is then incubated at room temperature for 5-10 minutes.
- Then 500µl of ice cold solution III is added to the mixture and mixed by inverting the tube.
- The mixture is incubated on ice for 10 minutes.
- The above step is followed by centrifugation of the mixture at 10.000 rpm for 5 minutes.
- The supernatant is transferred into a fresh tube.
- 400µl of phenol-chloroform mixture is added to the contents, and mixed well by inverting and incubating them at room temperature for 5 minutes.
- Again the mixture is centrifuged at 10.000 rpm for 5 minutes.

- The supernatant (viscous) is collected using cut tips and transferred to a fresh tube.
- 0.8 ml of isopropanol is added and mixed gently by inversion.
- The mixture is incubated at room temperature for 30 minutes.
- Centrifuge the contents at 10,000 rpm for 10 minutes.
- The supernatant is discarded after the centrifugation.
- This is followed by air drying for 5 minutes, and addition of 100µl of TE buffer or autoclaved distilled water to the pellet to resuspend the plasmid DNA.
- The DNA is run on the agarose gel.

AGAROSE GEL ELECTROPHORESIS

Introduction

Agarose gel electrophoresis is a powerful and widely used method that separates molecules on the basis of electrical charge, size, and shape. The method is particularly useful in separating charged biologically important molecules such as DNA (deoxyribonucleic acids), RNA (ribonucleic acids), and proteins. Agarose forms a gel like consistency when boiled and cooled in a suitable buffer.

Principle

The agarose gel contains molecule sized pores, acting like molecular sieves. The pores in the gel control the speed that molecules can move. DNA molecules possess a negative charge in their backbone structure due to the presence of PO_4^- groups thus this principle is exploited for its separation. Smaller molecules move through the pores more easily than larger ones. Conditions of charge, size, and shape interact with one another depending on the structure and composition of the molecules, buffer conditions, gel thickness, and voltage. Agarose gels are made with between 0.7% (provides good resolution of large 5–10 kb DNA fragments) and 2% (good resolution for small 0.2–1 kb fragments). The gel setup provides wells for loading DNA in to it. The loaded DNA molecules move towards the positively charged electrode (anode) and get separated along the length of the gel. Ethidium bromide (EtBr), a chromogen is added to the gel to visualize the separated DNA under UV transillumination. EtBr intercalates between the bases and glows when UV radiation is passed through the gel.

Purpose of gel loading buffer

The loading buffer gives colour and density to the sample to make it easy to load into the wells. Also, the dyes are negatively charged in neutral buffers and thus move in the same direction as the DNA during electrophoresis. This allows you to monitor the progress of the gel. The gel loading dye possesses bromophenol blue and xylene cyanol. Density is provided by glycerol or sucrose. Xylene xyanol gives a greenish blue colour while bromophenol blue provides bluish colored zone. The successful DNA run is determined by the presence of both the colored dye in the gel.

Materials Required

• Electrophoresis buffer: 1x TAE buffer

• Agarose ultra-pure (DNA graded)

• Electrophoresis tank, gel tray, sample comb and power supply

• Plastic or insulation tape

• Ethidium bromide: 10 mg /ml stock solution

• 5x Gel loading dye

• DNA marker solution, DNA sample and gloves.

PROCEDURE

Making a 1% Agarose Gel

• 0.5g of agarose is weighed and dissolved in 50ml of 1× TAE Buffer. (Note: A 250 ml of conical flask is used for the preparation to prevent wastage by spillage during the heating process.)

• The solution is heated over a hot plate to a boiling constituency marked with a clear solution.

• The solution is left to cool and 2µl of EtBr solution is added and mixed well by gently swirling.

• The solution is poured in the gel tray-comb set up. It is also made sure that the gel plates have been secured with tapes and the well combs are present prior to pouring.

• The solution is allowed to cool and harden to form gel.

2. Loading of Samples

• The gel is carefully transferred to the electrophoresis tank filled with 1× TAE buffer.

• The samples are prepared [8µl of DNA sample (0.1ug to 1ug) and 2ul of 5× gel loading dye].

• The comb is removed and the sample is loaded.

• Appropriate electrodes are connected to the power pack and run at 50-100 volts for 20 minutes.

• The progress of the gel is monitored with reference to tracking dye (Bromophenol blue). The run is stopped when the marker has run 3/4th of the gel.

3. Examining the gel

• The gel is placed on the UV-trans illuminator and checked for orange coloured bands in the gel.

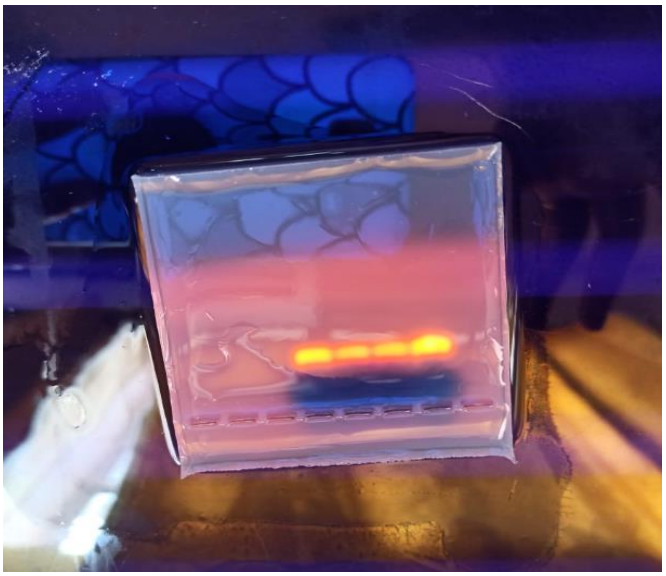


Fig. Agarose gel Electrophoresis

INTRODUCTION TO BIOINFORMATICS

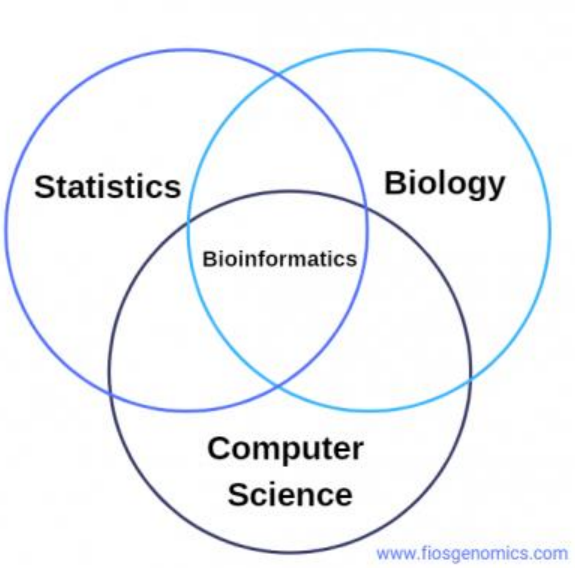
Definition

Bioinformatics is defined as the application of tools of computation and analysis to the capture and interpretation of biological data. It is an interdisciplinary field, which harnesses computer science, mathematics, physics, and biology.

Bioinformatics is essentially big data analysis for biological data sets. It requires computational and statistical analyses in order to extract meaning from biological data. Since this is the case, bioinformatics can also be used to refer to the development of the software and methods which are used to understand biological data. As you will now understand, bioinformatics is an interdisciplinary field where biology**,** computer science**,** and statistics meet.

Brief Summarization

- Bioinformatics is the application of tools of computation and analysis to the capture and interpretation of biological data
- Bioinformatics is essential for management of data in modern biology and medicine
- The bioinformatics toolbox includes computer software programs such as BLAST and Ensembl, which depend on the availability of the internet
- Analysis of genome sequence data, particularly the analysis of the human genome project, is one of the main achievements of bioinformatics to date
- Prospects in the field of bioinformatics include its future contribution to functional understanding of the human genome, leading to enhanced discovery of drug targets and individualised therapy.



Brief History

Paulien Hogeweg and Ben Hesper first coined the term 'bioinformatics' in the early 1970s and defined it as 'the study of informatic processes in biotic systems'[1]. However, before the term was coined the stage was set for bioinformatics to emerge as a new field of study in the 1960s when computational methods were applied to protein sequence analysis by Margaret Dayhoff. In fact, Dayhoff has been referred to as 'the mother and father of bioinformatics.

The Components of Bioinformatics

- Biology

  The statistical analyses you conduct will have no biological basis if you do not have an understanding of biology. While it might be useful to know that the Treatment A group has results showing higher values of Protein X than the Treatment B group, you need biological knowledge to understand the pathways or genes that cause that result. Is it due to an up-regulation of a certain pathway?

  You may find that without the biological knowledge to interpret your results, you know an ending without explanation and also without the method and mechanism of action.

- Computer Science

  Without computer science, analysis speed would be significantly slower. Bioinformaticians write software to help automate analyses (instead of manually

working through each one). Being able to combine hundreds of thousands of data points into analysis through coding allows for larger datasets to be created while still keeping the analysis time to a reasonable length. Depending on the size of the dataset, however, there can still be large computational time requirements, although it will be much reduced compared to a non-bioinformatic approach.

- Statistics

  In short, without statistics, there would be no in-depth analysis. Basic graphs may show that Treatment A has results with higher values of Protein X than Treatment B, but the actual significance of these results would be unknown. For example, is there a significant difference between A and B, or does it just look that way from the graph? New treatments need solid evidence behind them for approval and statistical analyses can prove the true benefits of a treatment.

Need for Bioinformatics

The field of bioinformatics has advanced considerably in the 50 years since the term was first coined. It has evolved to keep up with progress in molecular biology and computer science. The rise of the internet in the 1990s coupled with the creation of next-generation sequencing (NGS) technologies in the 2000s created a boom in the availability of biological data to be analyzed. In turn, this led to the swift production of new bioinformatics tools.

Nowadays, we can apply bioinformatic analyses to various biological data sets such as:

- Genome Sequence Data
- Gene Variation Data
- Gene Expression Data
- Single-Cell Data
- Proteomics Data
- Metabolomics Data
- Epigenetics Data

RESULTS

Day 1: Bacterial Growth

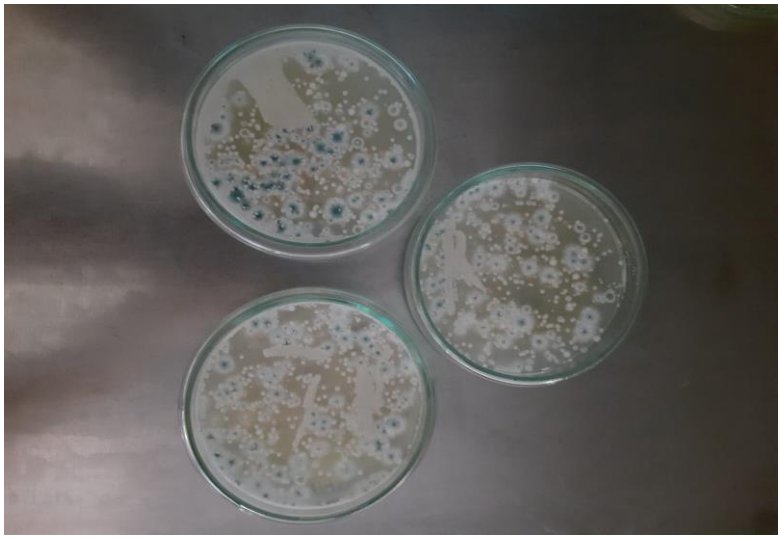


Fig: Bacterial growth is seen after incubated for 2 days.

Day 6: Purification of Microorganisms

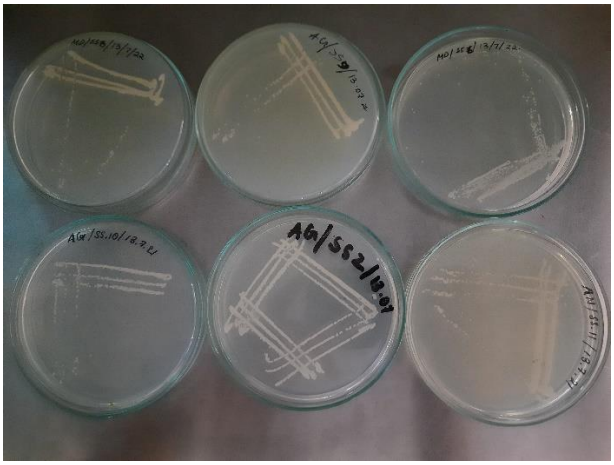


Fig: Streak plate method

In the first area of streaking, there is heavy growth with fused colonies, and gradually there are fewer colonies in subsequent streaks giving a few well-isolated colonies in the final streak.

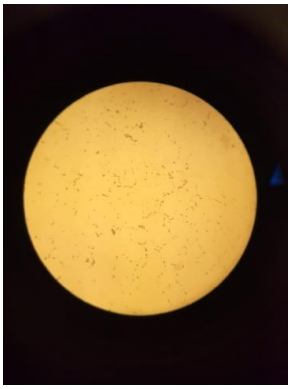
Day 7: Staining techniques

Gram staining of bacteria

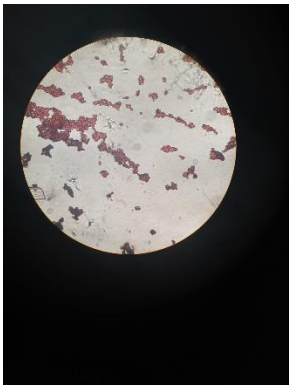
Table:

Culture	Color	Shape	Inference
SS1	Red	Coccoid	Gram negative
SS2	Red	Coccoid	Gram negative
SS3	Red	Coccoid	Gram negative
SS4	Red	Coccoid	Gram negative
SS5	Red	Coccoid	Gram negative
SS6	Red	Coccoid	Gram negative

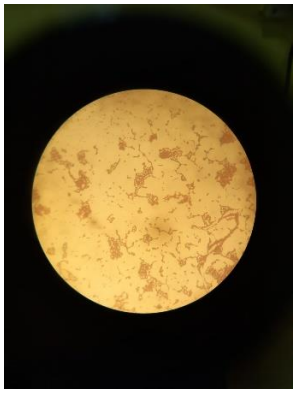
The slides which were observed under the microscope are as follows:



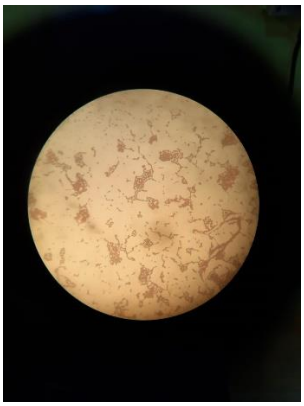
Slant culture 1



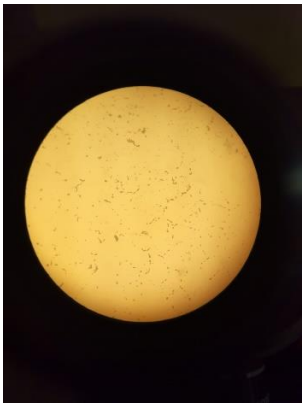
Slant culture 2



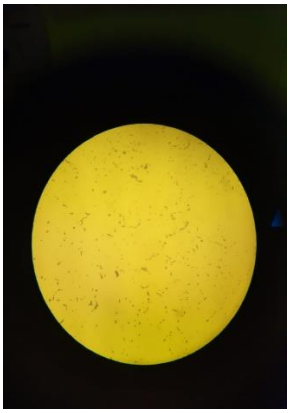
Slant culture 3



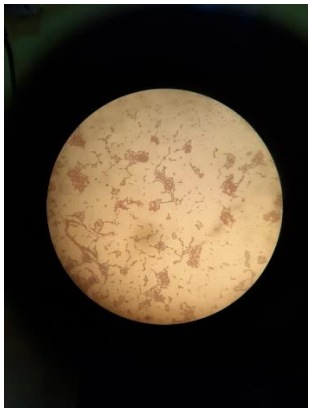
Slant culture 4



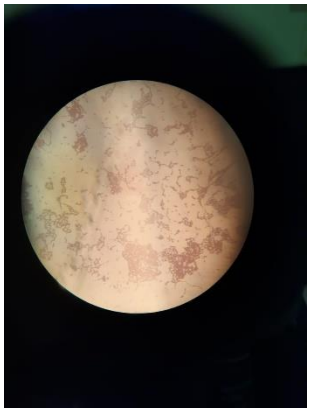
Slant culture 5



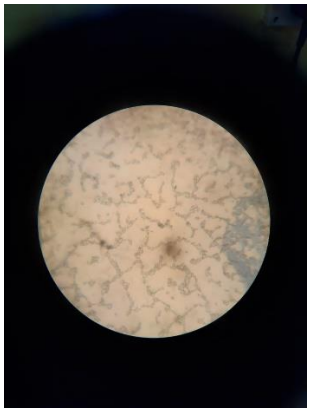
Slant culture 6



Slant culture 7



Slant culture 8



Slant culture 10

All the slides observed under the microscope were Gram negative and coccoid in shape.

Bacterial capsule training

The slides which were observed under the microscope are as follows:

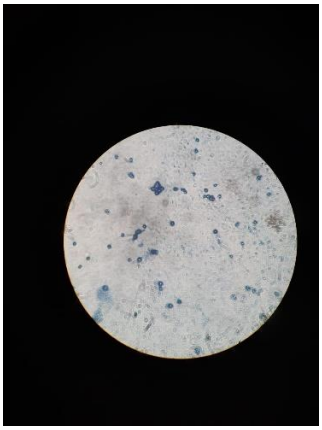


Fig: Slant culture 1

Fig: Slant culture 2

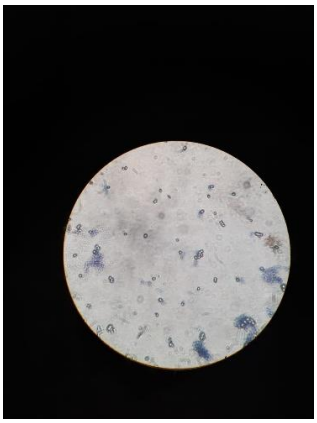


Fig: Slant culture 3

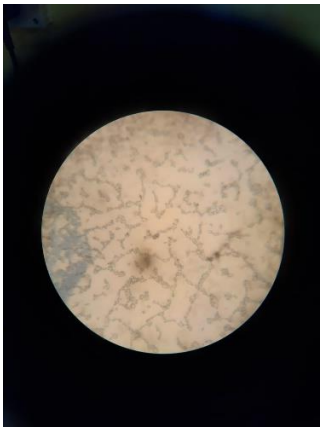


Fig: Slant culture 4

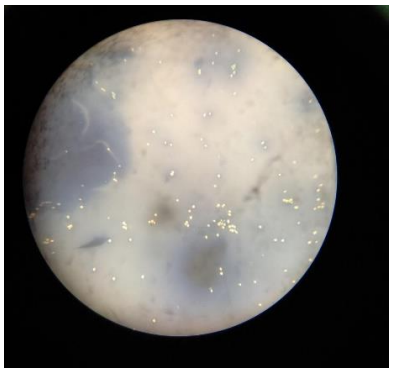


Fig: Slant culture 5

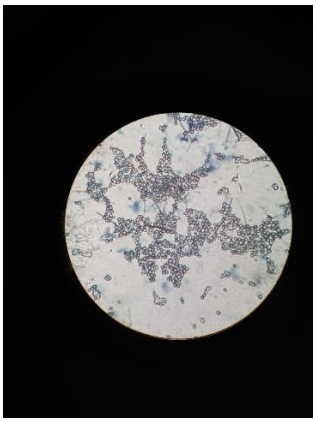


Fig: Slant culture 6

Slant culture 7

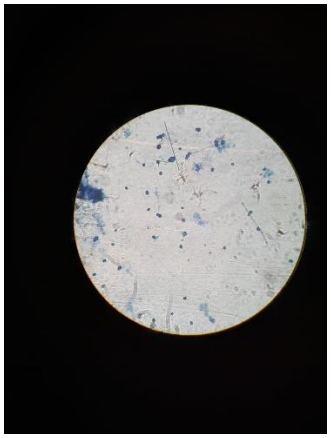


Fig: Slant culture 8

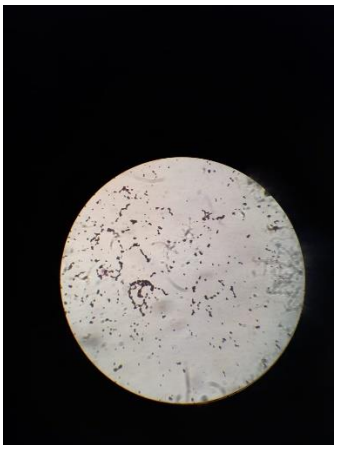


Fig: Slant culture 9

Fig:

All the samples observed under the microscope showed positive results for capsules.

Day 8: Biochemical Test

Catalase Test

Slant culture (SC)	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9
Oxidase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

Evolution of bubbles was observed in all the cultures.

Oxidase Test

Slant culture (SC)	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9
Oxidase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

A positive reaction is indicated by an intense deep-purple hue, appearing within 5-10 seconds.

Day 13: Agarose Gel Electrophoresis

The isolated genomic DNA as well as the Isolated Plasmid DNA were observed under a UV-trans illuminator after performing gel electrophoresis on both respectively.

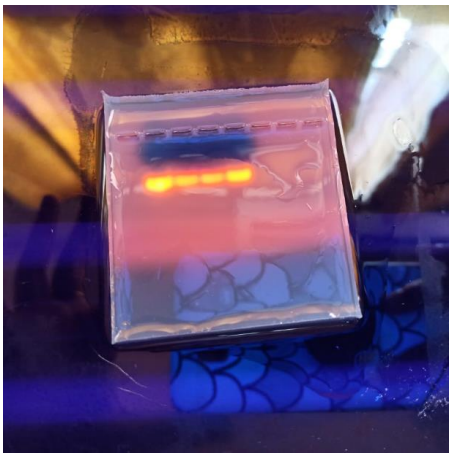


Fig. DNA bands observed under UV-trans illuminator after agarose gel electrophoresis.

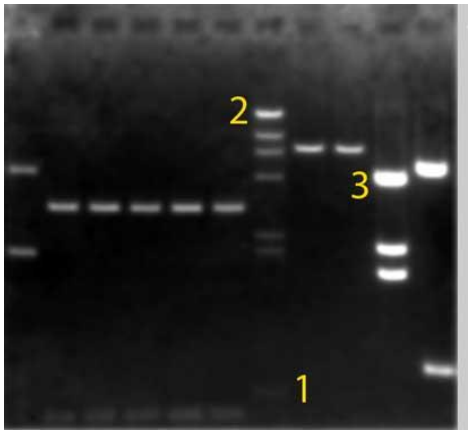


Fig. Plasmid DNA observed under UV-trans illuminator after performing agarose gel electrophoresis.

DISCUSSION

Microbiology is the study of small living things that are too small to be visible with the naked eye. These living things can be seen with the use of a microscope only. These life forms are called microorganisms of microbes. Microorganisms include bacteria, archaea, viruses, protozoa, microscopic fungi and yeasts, and microscopic algae. Microbiology research encompasses all aspects of these microorganisms such as their behavior, evolution, ecology, biochemistry, and physiology, along with the pathology of diseases that they cause. These microbes play crucial roles in nutrient cycling, biodegradation, climate change, food spoilage, the cause and control of disease, and biotechnology. They make up more than 60 percent of the Earth's living matter and scientists estimate that 2 to 3 billion species share the planet with us, also some microbial cells break down organic matter and recycle nutrients, such as carbon and nitrogen, that are necessary for the life on Earth. They can be used in many ways, making life-saving drugs, the manufacturing of biofuels, cleaning up of pollution and producing or processing of foods and drinks.

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CONTENTS: **Page no**

INSTRUMENTS HANDLED:

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Fig 1: Microwave

- **CENTRIFUGE-** A centrifuge is a device that uses centrifugal force to separate various components of a fluid.

  Centrifuge applies centrifugal force to separate suspended particles from a liquid or to separate liquids of

  different densities. These liquids can include body fluids (e.g. blood, serum, urine), commercial reagents, or combinations of the two with other additives. By creating forces many times greater than gravity, centrifuges can greatly accelerate separations that occur naturally as a result of density differences. Low speed centrifuges generally operate at up to 10,000 revolutions per minute (rpm) and may be non-refrigerated or refrigerated. High speed centrifuges generally operate at 10,000 to 30,000 rpm and some are refrigerated to cool the rotor chamber.

  The **relative centrifugal force (RCF)** or the **g force** is the radial force generated by the spinning rotor as expressed relative to the earth's gravitational force. The g force acting on particles is exponential to the speed of the rotation defined as **revolutions per minute (RPM).**

  G Force Formula:

  g Force (RCF) = $(RPM)^2 \times 1.118 \times 10^{-8} \times r$

Fig 2 : Centrifuge

- **WATER BATH –** A water bath is a laboratory equipment that is used to incubate samples at a constant temperature over a long period of time. Water bath is a preferred heat source for heating flammable chemicals instead of an open flame to prevent ignition. A water bath generally consists of a heating unit, a stainless-steel chamber that holds the water and samples and a control interface.

Fig 3 : Water Bath

STERILIZATION METHOD :

- **DRY HEAT**- Dry heat sterilization of an object is one of the earliest forms of sterilization practiced. It uses hot air that is either free from water vapor or has very little of it, where this moisture plays a minimal or no role in the process of sterilization. Dry heat destroys microorganisms by causing denaturation of proteins. Example- Hot Air Oven.

Fig 4 : Hot Air Oven

Hot air ovens are electrical devices which use dry heat to sterilize. They use a thermostat to control the temperature. Their double walled insulation keeps the heat in and conserves energy, the inner layer being a poor conductor and outer layer being metallic. They are widely used to sterilized articles that can withstand high temperatures and not get burnt, like glassware and powders. Linen gets burnt and surgical sharps lose their sharpness.

- **WET HEAT**- Moist heat sterilization is a procedure in which heated, high pressure steam is used to sterilize an object. This sterilization technique does not involve any toxic liquids or fumes, and it's relatively inexpensive, quick and effective in killing and eliminating potentially infectious bacteria, viruses and spores. Example- Autoclave.

An autoclave is a machine used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure and/or temperature. Autoclaves are used before surgical procedures to perform sterilization and in the chemical industry to cure coatings and vulcanize rubber and for hydrothermal synthesis. Many autoclaves are used to sterilize equipment and supplies by subjecting them to pressurized saturated steam at 121° C for around 30-60 minutes at a pressure of 15 psi depending on the size of the load and the contents.

Spores of *Geobacillus stearothermophilus* (formely) called *Bacillus stearothermophilus)* are the best indicator because they are resistant to steam. Their spores are killed in 12 minutes at 121°C. The Centers for Disease Control (CDC) recommends weekly autoclaving of a culture containing heat resistant endospores of *Geobacillus stearothermophilus,* to check autoclave performance. The spore strip and an ampule of medium enclosed in a soft plastic vial are available commercially. The vial is placed in the center of the material to be sterilized and is autoclaved. Then the inner ampule is broken releasing the medium and the whole container is incubate, if no growth appears in the autoclave culture sterilization is deemed effective.

Fig 5: Autoclave

- **FILTRATION**- Filtration is the preferred method of sterilizing heat-sensitive liquid and gases without exposure to denaturating heat . Rather than destroying contaminating microorganisms, it simply removes them. It is the method of choice for sterilizing antibiotic solutions, toxic chemicals, radioisotopes, vaccines and carbohydrates which are all heat sensitive.

- **SOLVENTS**- Ethanol is commonly used as a disinfectant , but isopropanol is a better solvent for fat and is probably a better option. Both solvents work by denaturing proteins through a process that requires water, so they must be diluted to 60-90% in water to be effective.

- **RADIATION STERILIZATION**- Radiation kills germs that can cause disease and neutralizes other harmful organisms. Sterilization with ionizing radiation inactivates microorganisms very efficiently and used for wrapping ensures that healthcare products are safe and can be relied upon. Example: Laminar Air Flow Cabinet (LAF).

Laminar air flow cabinet is a carefully enclosed bench, designed to prevent contamination of biological samples during dispensing of culture media and inoculation. Air is drawn through a filter and blown in a very smooth laminar flow towards the user. The cabinet is usually made in such a way that there are no gaps or joints where spores might collect. It has a UV-C germicidal lamp to sterilize the working area before use.

UV light is electromagnetic radiation with wavelengths shorter than visible light nut longer than X-rays. UV is categorized into several wavelength ranges, with short-wavelength UV (UV-C) considered “germicidal UV”. Wavelengths between about 200 nm and 300nm are strongly absorbed by nucleic acids. The absorbed energy can result in defects including pyrimidine dimers. The dimers can prevent replicatiom or can prevent the expression of necessary proteins, resulting in the death or inactivation of the organism.

There are two types of filter present in Laminar Air Flow:

1. **Pre-filter** : which removes coarse particle.
2. **Hepa filter** : which removes about 99.9% pf particles > 0.3µm.

Fig 6 : Laminar Air Flow Cabinet

BACTERIAL CELL CULTURE (E.Coli)

Bacterial culture is a method that allows the multiplication of bacterial cells in or on a culture medium under controlled laboratory conditions. The exact conditions required for optimal replication will depend on the target bacterial species.

GROWTH OF BACTERIA

MATERIALS REQUIRED:

1. A DH5-alpha ampicilin resistant strain
2. Luria Bertani Broth
   Composition :
   - **Tryptone**: used to provide essential amino acids such as peptides and peptones to the growing bacteria.
   - **Yeast extract**: used to provide a plethora of organic compounds helpful for bacterial growth.
   - **Sodium chloride**: Sodium ions for transport and osmotic balance are provided by sodium chloride.
   - Final pH (at 25°C) 7.5 ± 0.2
3. Ampicilin Antibiotic
4. Conical flask
5. Autoclave
6. Laminar air flow

PROCEDURE:

- 5g of Luria Bertani Broth powder is weighed out and mixed with 200 ml of water in a conical flask.
- It is then stirred until the powder has dissolved and cotton plug is used to seal the flask.
- Then the media is autoclave for 15-20 mins at 121°C. After that the media is kept in the Laminar air flow for 24 hours.

INCUBATING OF BACTERIA

MATERIALS REQUIRED:

1. Bunsen burner
2. Centrifuge tube
3. Incubator

PROCEDURE :

- The bench is sterilized with alcohol and the bunsen burner is lighted.
- In two centrifuge tube 5ml of LB media and 5µml of antibiotic along with 5µml of M4 and M10 bacterial culture respectively is added.
- It is then kept for incubation at 37°C for 16-20 hours.

PLASMID DNA ISOLATION

Plasmids are extra chromosomal DNA that replicates independently of the bacterial chromosome. The isolation of plasmid DNA from bacteria is a crucial technique in molecular biology and is an essential step in many procedures such as cloning, DNA sequencing, transfection and gene therapy.

MATERIALS REQUIRED:

1. Lysosome
2. RNase
3. Vortex
4. Isopropanol
5. NID extraction buffer

   Composition

   - 5ml of 1M EDTA
   - 50 ml of 100mM tris
   - 37.5 ml of 2M NH_4Cl
   - 5g Sucrose
   - 0.5ml of triton × 100
   - 20 µl of 10mg/ml lysosome
   - 2.5 µl of 20mg/ml of RNase

PROCEDURE :

- The cells are pellet downed (10000 rpm for 2 mins).
- Buffer along with 20µl of lysosome and 2.5µl of RNase is added and the vortexed to mix the cell properly.
- Then it is heated at 65°C for 10 mins. The suspension turns milkish white.
- It is centrifuged at 12000 rpm for 2 mins, the pellet is then pipetted out.
- 250µl of isopropanol is added to the supernatant.
- A ring like structure is observed.
- Again it is centrifuged at 12000 rpm for 12 mins.
- The supernatant is removed carefully and 250-300 µl of 70% ethanol is added.
- Again the sample is centrifuged at 10000 rpm for 12 mins.
- It is allowed to air dry.
- It is then dissolved in 20-25µl of Tris-EDTA buffer.

CALCULATIONS:

Lysosome (10mg/ml)	RNase (20mg/ml)
200ml = 2ml	200ml = 0.25 ml
1ml = 2/200ml = 0.01ml	1ml = 0.25/200ml
= 0.01 × 1000	= 1.25µl
= 10µl	
2ml = 20µl	2ml = 2.5µl

CONCLUSION- After the procedure is finished, we performed Agarose Gel Electrophoresis to check the isolated plasmid DNA bands.

ELECTROPHORESIS

Electrophoresis technique is used in laboratories to separate macromolecules based on size .The technique applies a negative charge so proteins or DNA move towards a positive charge .Electrophoresis occurs under the influence of electric field. The speed and mobility depends upon their molecular size to charge ratio. Heavier molecules tend to move slowly than light molecules.

AGAROSE GEL ELECTROPHORESIS - Agarose gel is used in movement of DNA molecule. DNA is negatively charged particle moves towards cathode which is positive in charge. A loading dye is used to track the movement of nucleic acids in gel.

Ethidium Bromide is a DNA intercalating dye that is added to the gel to visualize the DNA molecule and helps in the illumination of the DNA molecule under UV light.

MAKING OF AGAROSE GEL FOR ELECTROPHORESIS:-

- Required amount of Agarose is measured in the weighing balance with the help of a spatula.
- The agarose is mixed with 1x TAE buffer.
- Ethidium Bromide is a DNA intercalating dye that is added to the gel visualise the DNA molecule and helps in the illumination of the DNA molecule under UV light.
- 4-5 µl of EtBr (10mg/ml) is added on gel .
- Then the gel is poured on casting tray evenly. Make sure there is no formation of bubbles.
- After gel solidifies the tray is put inside the tank and buffer solution is added. Then the sample along with loading dye is put in the wells which are created by the comb.

- Ladder DNA is added in a well and then is ran. After about 45-60 mins, the samples that moved from the wells and formed bands are observed under UV radiations in a gel documentation system.

  Preparation of 50 X TAE Buffer Solution (1L) :

  - Tris free base = 242g
  - Disodium EDTA = 18.61g
  - Glacial acetic acid = 57.1 ml
  - Double distilled water = to make up 1L of the buffer

After the solution is prepared, the constituents are allowed to mix thoroughly in hot plate stirrer with addition of magnetic bead in the solution. After stirring is completed the solution is filtered with the vacuum pump and then the clear solution is stored for further use.

Preparation of 1 X TAE Buffer Solution (500ml)

N1V1=N2V2

Where N1 = 50N, N2 = 1N, V1 = ?, V2 = 500ml

Therefore, N1V1=N2V2

$50 \times V1 = 1 \times 500$

$V1 = 10ml$

10ml of 50× TAE + 490ml of water

Stock concentration of Ethidium bromide is 10mg/ml. After addition of 5µl in 100 ml of agarose solution the total concentration is :

N1V1=N2V2

Where N1 = 10mg/ml, V1 = 5µl, N2 = ?, V2 = 100ml

Therefore, N1V1=N2V2

$10mg/ml \times 5\mu l = N2 \times 100ml$

Or $\frac{10 \times 0.005}{100} = N2$

Or $N2 = 0.0005\ mg/ml = \frac{0.0005\ mg}{1ml} = \frac{0.0005 \times 1000}{1}$

$= 0.5\mu g/ml$ (Since 1000 µg = 1mg)

Composition of DNA gel-loading dye (10X)

1. 3.9 ml glycerol
2. 500µl 10% (w/v) SDS
3. 200µl 0.5M EDTA
4. 0.025g bromophenol blue
5. 0.025g xylene cyanol

Bringing to 10ml total volume with H2O.

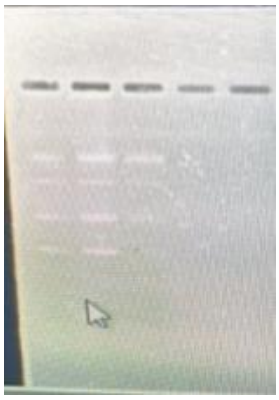


Fig 7: DNA bands observed

Fig 8: Gel doc system

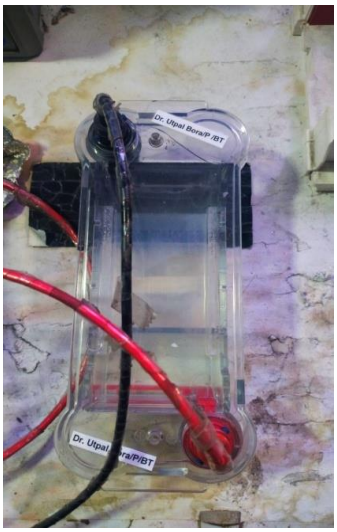


Fig 9: Agarose gel Electrophoresis set up

MICROBIAL CULTURE TECHNIQUES-

1. **Liquid Culture Media** - are sometimes referred as - "**broth**". Microbes grow faster in this media and grows properly. Microbe grows in qualitative product form. In this media bacteria grow uniformly producing general turbidity. Colonies can't be isolated because liquid media is not stable. Example: Nutrient broth
2. **Solid Culture Media** – Agar is the most commonly used solidifying agent. In this type of culture media, morphological structure of bacteria are studied by plating. It is for the isolation of bacteria as a pure culture on a solid medium. Colonies identification is done on this medium. Example : Nutrient agar

MICROBIAL PLATING TECHNIQUES –

1. **Spread Plate Method** – It is a technique to plate a liquid sample containing agar and bacteria so that the bacteria are easy to count and isolate. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate. It involves using a sterilized spreader with a smooth spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate. The plate needs to be dry at room temperature so that the agar can absorb the bacteria more readily.
2. **Streak Plate Method** – It is a microbial culture technique where microbiological culture technique, where a sample is spread in a petri dish in a form of a long, thin line over the surface of a solid media.
3. **Pour Plate Method** – The pour plate method is a microbiological laboratory technique for isolating and counting the viable microorganisms present in a liquid sample, which is added along with or before molten agar medium prior to its solidification. This technique is generally used to count viable microorganisms in the given sample by enumerating the total number of colony forming units (CFUs) within and/or on the surface of the solid medium. It is mostly used for enumerating bacteria; however, Actinobacteria, molds and yeasts can also be isolated and enumerated.

RESULTS:

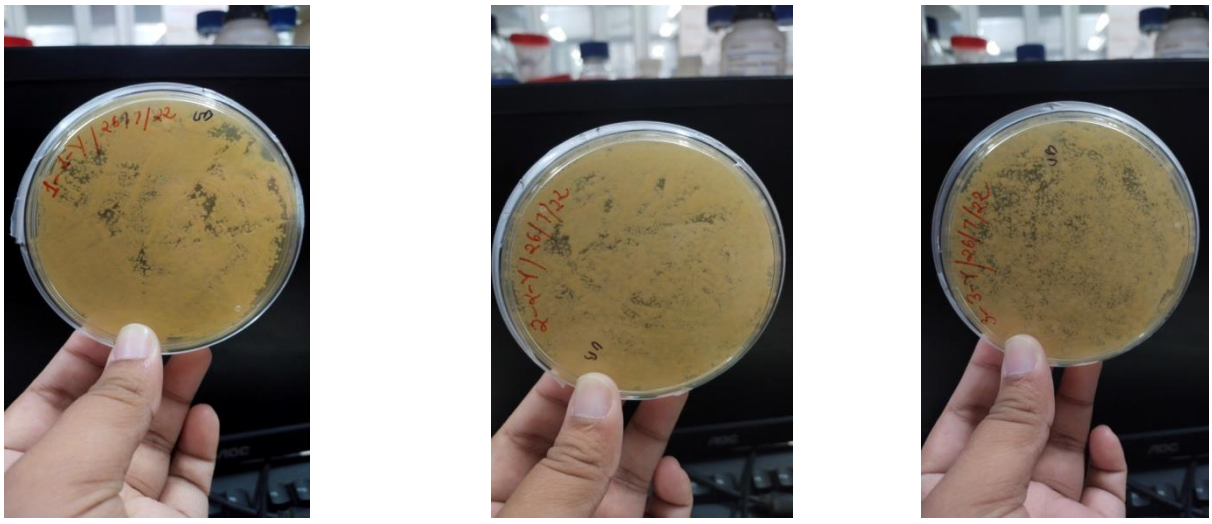
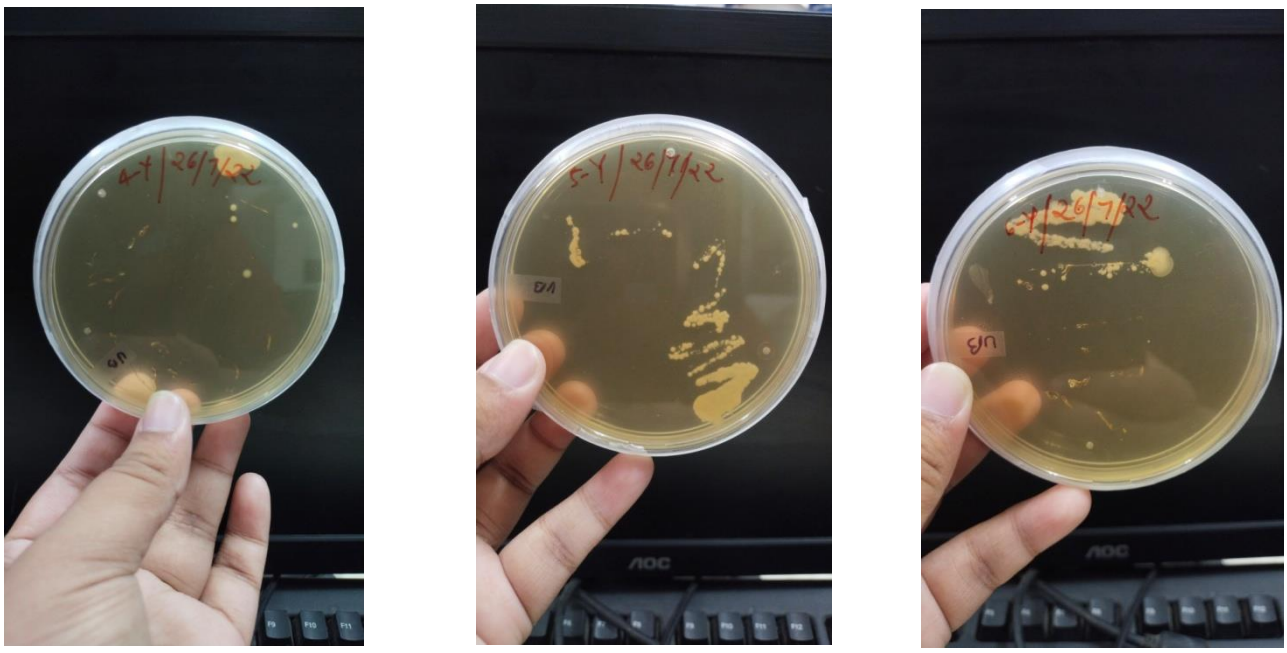


Fig 10: Results after spread plating technique



1:1 dilution factor 1:3 dilution factor 1:7 dilution factor

Fig 11: Results after streak plating technique

CULTURE OF YEAST :

Inoculation of yeast was done by **two types of methods:**

1. Liquid Culture of YEAST-

Composition of ingredients taken-For 100 ml water- Bacto Peptone -**2g**, yeast extract **1g**, dextrose-**2g.**

All the components were mixed with 100 ml of water in a conical flask. Then it was kept for sterilization in autoclave under 121°C, at 15 psi for 30-60 minutes. After sterilization process the equipments, along with the liquid media of yeast, all were again sterilized under **UV RADIATION**, i.e., under **LAMINAR AIR FLOW** for 15 mins. After this process ***Saccharomyces Cerevisiae*** yeast powder was added into the liquid media of yeast**.** After this process liquid media of yeast was kept under Incubator 30°C for inoculation.

2. Solid Culture of Yeast – **AGAR** is used **solidifying agent.** For preparing the media above process is followed. After this procedure liquid agar media**,** was poured in petri dish and kept for drying. After it gets dried, Yeast sample was poured into the petri dish and kept inside the incubator at 30°C.

This process was followed by **serial dilution .**

Serial dilution is a laboratory technique, in which a stepwise dilution process is performed on a solution with an associated dilution factor. In laboratory this method is used to decrease the counts of viable cells within a culture to simplify the operation. The main purpose of serial dilution technique is to find out the concentration or the cell counts of an anonymous sample by counting the number of colonies that are cultured from the serial dilutions of the sample.

Cryopreservation of yeast –

A method is described that allows a wide range of **yeast species** to be store in **glycerol,** which can be used for further experiments. It can be used for several years. Glycerol functions as preservation agent.

Glycerol Stock Preparation:

1. **400µl of Glycerol + 400µl of yeast extract** were mixed with micropipette inside **cryo preservation vials.**
2. **Glycerol stock** was stored in deep freeze in -80°C.

Pure Colony Isolation:

Pure colony is form by **spread plating technique**. It is done by the process-

1. **For 100 ml, Bacto peptone – 2g**
2. **Yeast extract-1g**
3. **Dextrose-2g**
4. **100 ml** of water was added
5. **Yeast media was kept inside autoclave for sterilization.**
6. After autoclaving process colonies from the petri dish were put into the conical flask and stored inside incubator, which can be used for further future experiments.

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CONTENTS: **Page no**

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- **WET HEAT**- Moist heat sterilization is a procedure in which heated, high pressure steam is used to sterilize an object. This sterilization technique does not involve any toxic liquids or fumes, and it's relatively inexpensive, quick and effective in killing and eliminating potentially infectious bacteria, viruses and spores. Example- Autoclave.

An autoclave is a machine used to carry out industrial and scientific processes requiring elevated temperature and pressure in relation to ambient pressure and/or temperature. Autoclaves are used before surgical procedures to perform sterilization and in the chemical industry to cure coatings and vulcanize rubber and for hydrothermal synthesis. Many autoclaves are used to sterilize equipment and supplies by subjecting them to pressurized saturated steam at 121° C for around 30-60 minutes at a pressure of 15 psi depending on the size of the load and the contents.

Spores of *Geobacillus stearothermophilus* (formely) called *Bacillus stearothermophilus)* are the best indicator because they are resistant to steam. Their spores are killed in 12 minutes at 121°C. The Centers for Disease Control (CDC) recommends weekly autoclaving of a culture containing heat resistant endospores of *Geobacillus stearothermophilus,* to check autoclave performance. The spore strip and an ampule of medium enclosed in a soft plastic vial are available commercially. The vial is placed in the center of the material to be sterilized and is autoclaved. Then the inner ampule is broken releasing the medium and the whole container is incubate, if no growth appears in the autoclave culture sterilization is deemed effective.

Fig 5: Autoclave

- **FILTRATION**- Filtration is the preferred method of sterilizing heat-sensitive liquid and gases without exposure to denaturating heat . Rather than destroying contaminating microorganisms, it simply removes them. It is the method of choice for sterilizing antibiotic solutions, toxic chemicals, radioisotopes, vaccines and carbohydrates which are all heat sensitive.

- **SOLVENTS**- Ethanol is commonly used as a disinfectant , but isopropanol is a better solvent for fat and is probably a better option. Both solvents work by denaturing proteins through a process that requires water, so they must be diluted to 60-90% in water to be effective.

- **RADIATION STERILIZATION**- Radiation kills germs that can cause disease and neutralizes other harmful organisms. Sterilization with ionizing radiation inactivates microorganisms very efficiently and used for wrapping ensures that healthcare products are safe and can be relied upon. Example: Laminar Air Flow Cabinet (LAF).

Laminar air flow cabinet is a carefully enclosed bench, designed to prevent contamination of biological samples during dispensing of culture media and inoculation. Air is drawn through a filter and blown in a very smooth laminar flow towards the user. The cabinet is usually made in such a way that there are no gaps or joints where spores might collect. It has a UV-C germicidal lamp to sterilize the working area before use.

UV light is electromagnetic radiation with wavelengths shorter than visible light nut longer than X-rays. UV is categorized into several wavelength ranges, with short-wavelength UV (UV-C) considered “germicidal UV”. Wavelengths between about 200 nm and 300nm are strongly absorbed by nucleic acids. The absorbed energy can result in defects including pyrimidine dimers. The dimers can prevent replicatiom or can prevent the expression of necessary proteins, resulting in the death or inactivation of the organism.

There are two types of filter present in Laminar Air Flow:

1. **Pre-filter** : which removes coarse particle.
2. **Hepa filter** : which removes about 99.9% pf particles > 0.3µm.

Fig 6 : Laminar Air Flow Cabinet

BACTERIAL CELL CULTURE (E.Coli)

Bacterial culture is a method that allows the multiplication of bacterial cells in or on a culture medium under controlled laboratory conditions. The exact conditions required for optimal replication will depend on the target bacterial species.

GROWTH OF BACTERIA

MATERIALS REQUIRED:

1. A DH5-alpha ampicilin resistant strain
2. Luria Bertani Broth
   Composition :
   - **Tryptone**: used to provide essential amino acids such as peptides and peptones to the growing bacteria.
   - **Yeast extract**: used to provide a plethora of organic compounds helpful for bacterial growth.
   - **Sodium chloride**: Sodium ions for transport and osmotic balance are provided by sodium chloride.
   - Final pH (at 25°C) 7.5 ± 0.2
3. Ampicilin Antibiotic
4. Conical flask
5. Autoclave
6. Laminar air flow

PROCEDURE:

- 5g of Luria Bertani Broth powder is weighed out and mixed with 200 ml of water in a conical flask.
- It is then stirred until the powder has dissolved and cotton plug is used to seal the flask.
- Then the media is autoclave for 15-20 mins at 121°C. After that the media is kept in the Laminar air flow for 24 hours.

INCUBATING OF BACTERIA

MATERIALS REQUIRED:

1. Bunsen burner
2. Centrifuge tube
3. Incubator

PROCEDURE :

- The bench is sterilized with alcohol and the bunsen burner is lighted.
- In two centrifuge tube 5ml of LB media and 5µml of antibiotic along with 5µml of M4 and M10 bacterial culture respectively is added.
- It is then kept for incubation at 37°C for 16-20 hours.

PLASMID DNA ISOLATION

Plasmids are extra chromosomal DNA that replicates independently of the bacterial chromosome. The isolation of plasmid DNA from bacteria is a crucial technique in molecular biology and is an essential step in many procedures such as cloning, DNA sequencing, transfection and gene therapy.

MATERIALS REQUIRED:

1. Lysosome
2. RNase
3. Vortex
4. Isopropanol
5. NID extraction buffer

   Composition

   - 5ml of 1M EDTA
   - 50 ml of 100mM tris
   - 37.5 ml of 2M NH_4Cl
   - 5g Sucrose
   - 0.5ml of triton × 100
   - 20 µl of 10mg/ml lysosome
   - 2.5 µl of 20mg/ml of RNase

PROCEDURE :

- The cells are pellet downed (10000 rpm for 2 mins).
- Buffer along with 20µl of lysosome and 2.5µl of RNase is added and the vortexed to mix the cell properly.
- Then it is heated at 65°C for 10 mins. The suspension turns milkish white.
- It is centrifuged at 12000 rpm for 2 mins, the pellet is then pipetted out.
- 250µl of isopropanol is added to the supernatant.
- A ring like structure is observed.
- Again it is centrifuged at 12000 rpm for 12 mins.
- The supernatant is removed carefully and 250-300 µl of 70% ethanol is added.
- Again the sample is centrifuged at 10000 rpm for 12 mins.
- It is allowed to air dry.
- It is then dissolved in 20-25µl of Tris-EDTA buffer.

CALCULATIONS:

Lysosome (10mg/ml)	RNase (20mg/ml)
200ml = 2ml	200ml = 0.25 ml
1ml = 2/200ml = 0.01ml	1ml = 0.25/200ml
= 0.01 × 1000	= 1.25µl
= 10µl	
2ml = 20µl	2ml = 2.5µl

CONCLUSION- After the procedure is finished, we performed Agarose Gel Electrophoresis to check the isolated plasmid DNA bands.

ELECTROPHORESIS

Electrophoresis technique is used in laboratories to separate macromolecules based on size .The technique applies a negative charge so proteins or DNA move towards a positive charge .Electrophoresis occurs under the influence of electric field. The speed and mobility depends upon their molecular size to charge ratio. Heavier molecules tend to move slowly than light molecules.

AGAROSE GEL ELECTROPHORESIS - Agarose gel is used in movement of DNA molecule. DNA is negatively charged particle moves towards cathode which is positive in charge. A loading dye is used to track the movement of nucleic acids in gel.

Ethidium Bromide is a DNA intercalating dye that is added to the gel to visualize the DNA molecule and helps in the illumination of the DNA molecule under UV light.

MAKING OF AGAROSE GEL FOR ELECTROPHORESIS:-

- Required amount of Agarose is measured in the weighing balance with the help of a spatula.
- The agarose is mixed with 1x TAE buffer.
- Ethidium Bromide is a DNA intercalating dye that is added to the gel visualise the DNA molecule and helps in the illumination of the DNA molecule under UV light.
- 4-5 µl of EtBr (10mg/ml) is added on gel .
- Then the gel is poured on casting tray evenly. Make sure there is no formation of bubbles.
- After gel solidifies the tray is put inside the tank and buffer solution is added. Then the sample along with loading dye is put in the wells which are created by the comb.

- Ladder DNA is added in a well and then is ran. After about 45-60 mins, the samples that moved from the wells and formed bands are observed under UV radiations in a gel documentation system.

  Preparation of 50 X TAE Buffer Solution (1L) :

  - Tris free base = 242g
  - Disodium EDTA = 18.61g
  - Glacial acetic acid = 57.1 ml
  - Double distilled water = to make up 1L of the buffer

After the solution is prepared, the constituents are allowed to mix thoroughly in hot plate stirrer with addition of magnetic bead in the solution. After stirring is completed the solution is filtered with the vacuum pump and then the clear solution is stored for further use.

Preparation of 1 X TAE Buffer Solution (500ml)

N1V1=N2V2

Where N1 = 50N, N2 = 1N, V1 = ?, V2 = 500ml

Therefore, N1V1=N2V2

$50 \times V1 = 1 \times 500$

$V1 = 10ml$

10ml of 50× TAE + 490ml of water

Stock concentration of Ethidium bromide is 10mg/ml. After addition of 5µl in 100 ml of agarose solution the total concentration is :

N1V1=N2V2

Where N1 = 10mg/ml, V1 = 5µl, N2 = ?, V2 = 100ml

Therefore, N1V1=N2V2

$10mg/ml \times 5\mu l = N2 \times 100ml$

Or $\frac{10 \times 0.005}{100} = N2$

Or $N2 = 0.0005\ mg/ml = \frac{0.0005\ mg}{1ml} = \frac{0.0005 \times 1000}{1}$

$= 0.5\mu g/ml$ (Since 1000 µg = 1mg)

Composition of DNA gel-loading dye (10X)

1. 3.9 ml glycerol
2. 500µl 10% (w/v) SDS
3. 200µl 0.5M EDTA
4. 0.025g bromophenol blue
5. 0.025g xylene cyanol

Bringing to 10ml total volume with H2O.

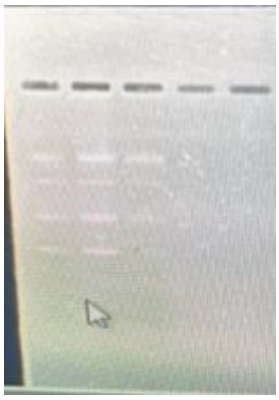


Fig 7: DNA bands observed

Fig 8: Gel doc system

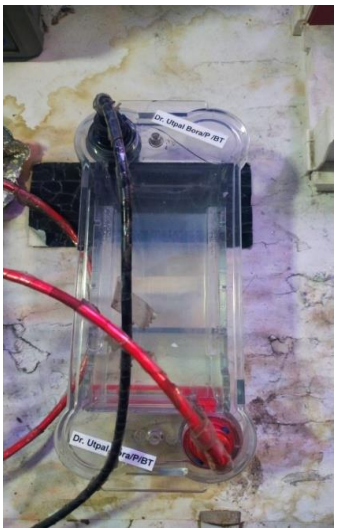


Fig 9: Agarose gel Electrophoresis set up

MICROBIAL CULTURE TECHNIQUES-

1. **Liquid Culture Media** - are sometimes referred as - "**broth**". Microbes grow faster in this media and grows properly. Microbe grows in qualitative product form. In this media bacteria grow uniformly producing general turbidity. Colonies can't be isolated because liquid media is not stable. Example: Nutrient broth
2. **Solid Culture Media** – Agar is the most commonly used solidifying agent. In this type of culture media, morphological structure of bacteria are studied by plating. It is for the isolation of bacteria as a pure culture on a solid medium. Colonies identification is done on this medium. Example : Nutrient agar

MICROBIAL PLATING TECHNIQUES –

1. **Spread Plate Method** – It is a technique to plate a liquid sample containing agar and bacteria so that the bacteria are easy to count and isolate. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate. It involves using a sterilized spreader with a smooth spreader with a smooth surface made of metal or glass to apply a small amount of bacteria suspended in a solution over a plate. The plate needs to be dry at room temperature so that the agar can absorb the bacteria more readily.
2. **Streak Plate Method** – It is a microbial culture technique where microbiological culture technique, where a sample is spread in a petri dish in a form of a long, thin line over the surface of a solid media.
3. **Pour Plate Method** – The pour plate method is a microbiological laboratory technique for isolating and counting the viable microorganisms present in a liquid sample, which is added along with or before molten agar medium prior to its solidification. This technique is generally used to count viable microorganisms in the given sample by enumerating the total number of colony forming units (CFUs) within and/or on the surface of the solid medium. It is mostly used for enumerating bacteria; however, Actinobacteria, molds and yeasts can also be isolated and enumerated.

RESULTS:

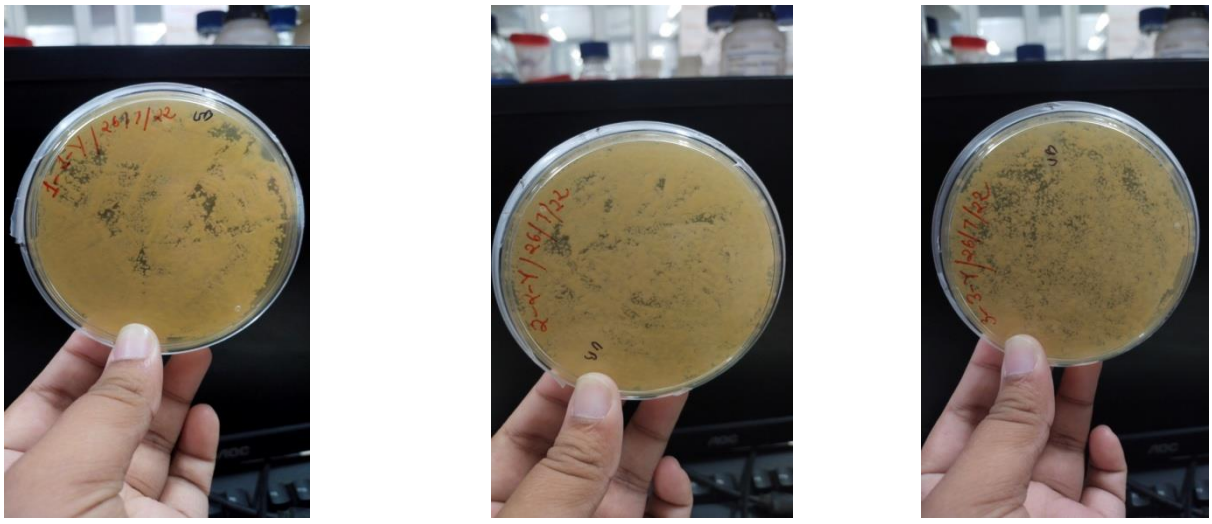
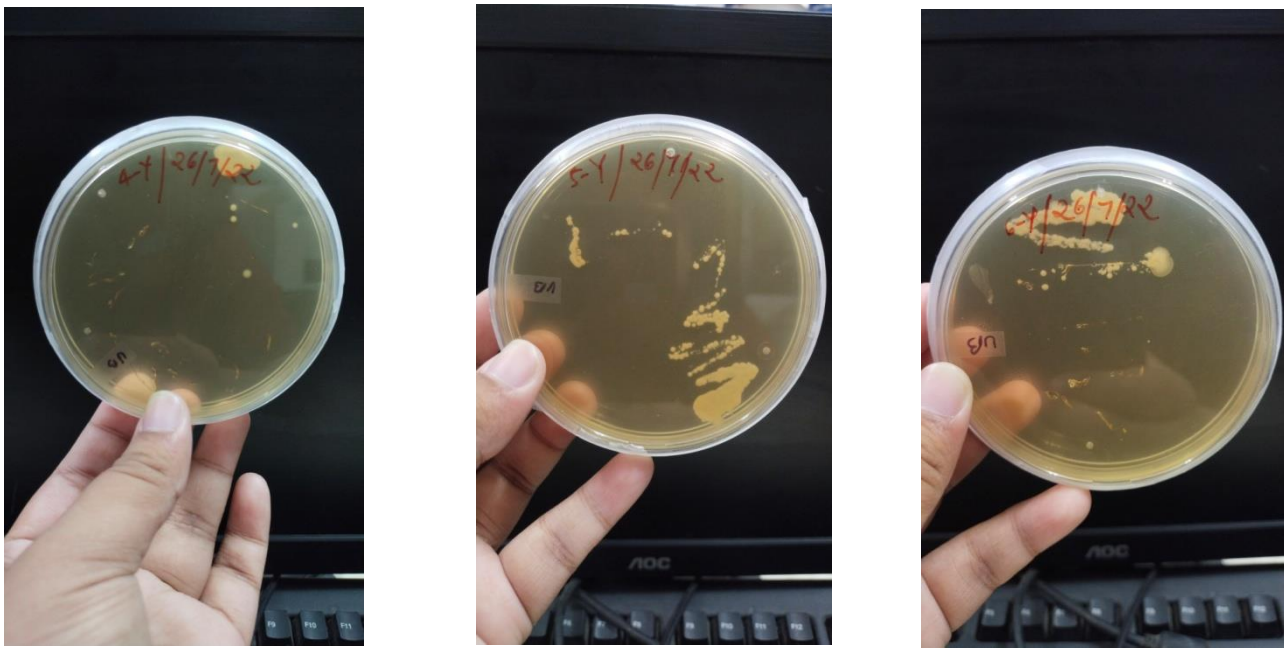


Fig 10: Results after spread plating technique



1:1 dilution factor 1:3 dilution factor 1:7 dilution factor

Fig 11: Results after streak plating technique

CULTURE OF YEAST :

Inoculation of yeast was done by **two types of methods:**

1. Liquid Culture of YEAST-

Composition of ingredients taken-For 100 ml water- Bacto Peptone -**2g**, yeast extract **1g**, dextrose-**2g.**

All the components were mixed with 100 ml of water in a conical flask. Then it was kept for sterilization in autoclave under 121°C, at 15 psi for 30-60 minutes. After sterilization process the equipments, along with the liquid media of yeast, all were again sterilized under **UV RADIATION**, i.e., under **LAMINAR AIR FLOW** for 15 mins. After this process ***Saccharomyces Cerevisiae*** yeast powder was added into the liquid media of yeast**.** After this process liquid media of yeast was kept under Incubator 30°C for inoculation.

2. Solid Culture of Yeast – **AGAR** is used **solidifying agent.** For preparing the media above process is followed. After this procedure liquid agar media**,** was poured in petri dish and kept for drying. After it gets dried, Yeast sample was poured into the petri dish and kept inside the incubator at 30°C.

This process was followed by **serial dilution .**

Serial dilution is a laboratory technique, in which a stepwise dilution process is performed on a solution with an associated dilution factor. In laboratory this method is used to decrease the counts of viable cells within a culture to simplify the operation. The main purpose of serial dilution technique is to find out the concentration or the cell counts of an anonymous sample by counting the number of colonies that are cultured from the serial dilutions of the sample.

Cryopreservation of yeast –

A method is described that allows a wide range of **yeast species** to be store in **glycerol,** which can be used for further experiments. It can be used for several years. Glycerol functions as preservation agent.

Glycerol Stock Preparation:

1. **400µl of Glycerol + 400µl of yeast extract** were mixed with micropipette inside **cryo preservation vials.**
2. **Glycerol stock** was stored in deep freeze in -80°C.

Pure Colony Isolation:

Pure colony is form by **spread plating technique**. It is done by the process-

1. **For 100 ml, Bacto peptone – 2g**
2. **Yeast extract-1g**
3. **Dextrose-2g**
4. **100 ml** of water was added
5. **Yeast media was kept inside autoclave for sterilization.**
6. After autoclaving process colonies from the petri dish were put into the conical flask and stored inside incubator, which can be used for further future experiments.

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Training Report

On

"A study on Plant Tissue Culture"

At

DEPT. OF FOREST & ENVIRONMENT SILVICULTURE DIVISION
TISSUE CULTURE LABORATORY

4 MILE, UPPER SHILLONG, SHILLONG

Submitted by

Jasmine Jamatia

Department of Biotechnology

St. Edmund's College

Under the Guidance of

Mrs. Badawanshisha chyne *Mr. Rapki-o Sumer*

ACKNOWLEDGEMENT

For the successful completion of the summer training, I would to extend my thanks and gratitude to our head of department Dr. Samrat Adhikari (Dept. of Biotechnology) who gave me this wonderful opportunity to do this training at Department of silviculture and also to the teachers of our department for supporting me direct or indirectly for the fulfillment of this desertion.

I would also like to acknowledge my research guides Ms. Badawanshisha Chyne and Mr. Rapki- o for their continuous guidance during the training.

I would also like to express my gratitude towards the staff in department of silviculture for allowing me to use the laboratory, their lab equipment and also for helping me in many ways.

Jasmine Jamatia

BSc 5th semester

Dept. of Biotechnology

CONTENT

INTRODUCTION

The training was conducted in Silviculture Division, Tissue Culture Laboratory under the Department of Forest & Environment is situated at 4 ½ mile, Upper Shillong, Shillong. The main objective of the Division is Mapping and Inventorisation of the natural resources of the State for utilization in planning and focusing towards scientific management and exploitation for the ultimate object of sustain yield. The Division also actively engaged in implementing the schemes/works under the Intensification of Forest Management, and also collection of field data like location of plantations, nurseries, beats, ranges, boundary of Reserve Forests, Protected Forests, National Parks and sanctuaries, macro level mapping and preparation of management plan, macro level survey and inventory of Sacred Groves in the different Districts with the main objectives to protect and conserve the Sacred Groves of the State

Micropropagation or tissue culture is the practice of rapidly multiplying plant stock material to produce many progeny plants, using modern plant tissue culture methods.

Micropropagation is used to multiply a wide variety of plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from seedless plants, plants that do not respond well to vegetative reproduction or where micropropagation is the cheaper means of propagating (e.g. Orchids) Cornell University botanist Frederick Campion Steward discovered and pioneered micropropagation and plant tissue culture in the late 1950s and early 1960s.

In short, steps of micropropagation can be divided into 4 stages.

1. Selection of mother plant
2. Multiplication
3. Rooting and acclimatizing
4. Transfer new plant to soil

Date: 11th July 2022 and 25th July 2022

Hardening

This is a method in which the tissue culture plants developed in artificial media are habituated to grow in natural environment. Firstly, these plants are taken out from nutrient media and washed thoroughly with water. Then these plants are grown in netted plastic pots filled with liquid nutrient medium and kept in mist chamber for 6 – 8 weeks.

Fig. Plants taken out from nutrient media

Fig. Holes poked on plastic cups

This is called Primary hardening. Afterwards the plants are transferred to polybags filled with potting mixture and grown under shaded house for 6 – 8 weeks. This is called Secondary hardening. After secondary hardening the plants are suitable for growing in farmer's fields.

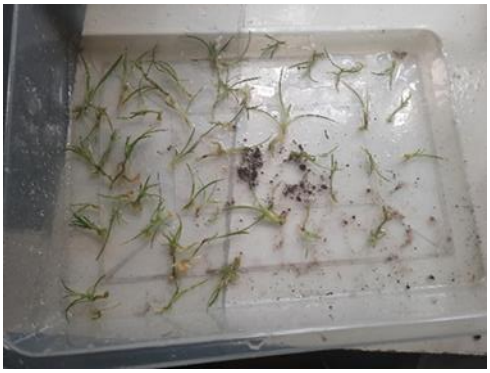

Fig. plants are washed and media is removed

Fig. Plants transferred to potting mixture

Date: 12th July 2022,18th July 2022 and 21st July 2022

Preparation of Plant tissue Culture Stock Solutions

Composition of MS media

Constituent	*Amount(mg/L)*	*For 1L (g/L)*
A. Macro Salts		
NH_4NO_3	1650 x 10 = 16500	16.5
KNO_3	190 x 10 = 1900	19
KH_2PO_4	170 x 10 = 1700	1.7
$MgSO_4.7H_2O$	370 x 10 = 3700	3.7
B. Calcium stock		
$Cacl_2.2H_2O$	440 x 1O = 4400	4.4
C. Micro Salts		
H_3BO_3	6.2 x 100 = 620	0.62
$MnSO_4.7H_2O$	16.9 x 100 =1690	1.69
$ZnSO_4.7H_2O$	8.6 x 100 = 860	0.86
$CuSO_4.5H_2O$	0.025 x 100 = 2.5	0.0025
$Cocl_2.6H_2O$	0.025 x 100 = 2.5	0.0025
KI	0.083 x 100 = 8.3	0.0083
$Na_2MoO_4.2H_2O$	0.25 x 100 = 2.5	0.0025
D. Iron stock		
$FeSO_4.7H_2O$	27.8 x 10 = 278	0.278
Na_2EDTA	37.3 x 10 = 373	0.373
E. Organic stock		

Pyridine-HCl	0.50 x 100 = 50	0.05
Nicotinic Acid	0.50 x 100 = 50	0.05
Thiamine HCl	0.1 x 100 = 10	0.01
Glycine	2 x 100 = 200	0.2

a. Preparation of macro stock (MS-I)

Take 500 ml double distilled water in a 2.0-liter beaker, weigh, add and keep on dissolving each salt sequentially in a descending order as listed above and finally make up the volume to 1000 ml by distilled water.

b. Preparation of calcium stock (MS-II)

Take 500 ml double distilled water in a 2.0-liter beaker, weigh and add $CaCl_2$ .2H2O, dissolve by stirring, and finally make up the volume to 1000 ml by distilled water.

c. Preparation of micro stock (MS-III)

Take 500 ml of double distilled water in a 2.0-liter beaker, weigh, add and keep on dissolving each salt sequentially in descending order as listed above, and finally make up the volume to 1000 ml by distilled water.

d. Preparation of iron stock (MS-IV)

Take 500 ml double distilled water in a 2.0-liter amber colored bottler. Now weigh and add Na_2EDTA and stir; after Na_2EDTA has been dissolved, add gradually $FeSO_4.7H_2O$ while still under mild stirring. This will yield a clear yellow solution.

e. Preparation of organic stock (MS-V)

Take 500 ml double distilled water in a 2.0-liter beaker, keep on adding and dissolving each salts sequentially in descending order as listed above, and finally make up the volume to 1000 ml by adding distilled water.

Fig. Prepared stock for MS media

Date: 13th July 2022,15th July 2022 and 21st July 2022

Preparation of MS Media

MATERIALS REQUIRED:

1. Stocks
2. Growth regulators (IAA, NAA, BAP)
3. Marker
4. Glass bottles
5. Sucrose
6. Agar
7. Inositol
8. Large Conical flask
9. Heater or induction
10. Spatula
11. Weighing machine
12. Paper and rubber bands
13. Cloth
14. Micropipette

PROCEDURE

- MS + 0.1 BAP + 0.1 IAA
- MS + 0.1 BAP + 0.1NAA
- MS + 0.1 IAA
- MS + 0.1 NAA
- MS + 0.1 IAA O.1 NAA

In a Conical flask adding

- Macro stock = 100 ml
- Micro stock 10 ml
- Cacl2.2H2O = 100ml
- FeSO4.NaEDTA= 100 ml
- Organic =10ml
- Myoinositol = 0.1g
- Sucrose = 30 g

1. 0.1 BAP and 0.1 IAA was added and the liquid was mixed properly and the PH which must be between 5.6 to 5.8 once done the media was poured into the measuring cylinder then distilled water as added up to 1000ml then the liquid was poured back to the flask then kept it on the induction. Once it heats up Agar about 8 g (and 7.5 g for *Nepenthes)* was added and Shaked vigorously.
2. After that the media was poured into the glass bottles of equal quantity and covered then again covered with the paper.
3. Also following the same procedures for the rest.
4. Once done with all the media is kept into the autoclave.

Fig. Sucrose added to a conical flask

Fig. pH measure between 5.6 and 5.8

Date:19.07.2022

SURFACE STERILIZATION OF ORCHID SEEDS:

1. The seeds were washed with soap and water.
2. They were then soaked in a solution of 800ml distilled water and 10ml of Dettol in a beaker and were kept for 15-20mins.
3. The seeds were then washed under running water followed by washing it with distilled water.

Fig: Sterilization of Orchid seeds.

INOCULATION OF ORCHID SEEDS:

1. This step is proceeded by further sterilizing the tools used viz., scalpel and forceps by heating them under the spirit lamp.
2. Inoculation of the seeds were done inside a laminar flow where the seeds were dissected into two parts using a scalpel and measures were taken to not contaminate the seeds by not touching the seeds directly with bare hands but instead forceps were used to hold the seed.
3. The inner part of the seed was then used for propagation by carefully spreading them on the required media for its growth by gently tapping the

tweezer that is used to pick the seeds on the glass bottle that contains the required medium for the growth of the seed.

4. The cap as well as the bottle was heat sterilized for few seconds before covering the bottle.
5. After all the bottles were filled, they were taken to the culture room where they are kept to grow.

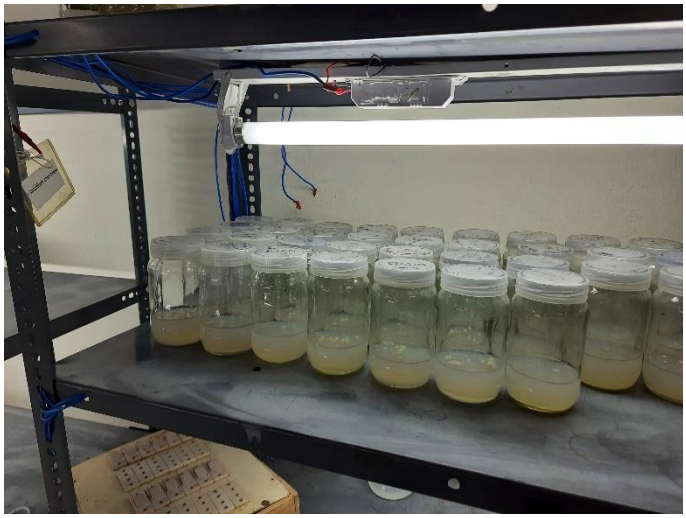

Fig. incubation of orchids

Date:20th July,2022

SURFACE STERILISATION FOR *Nepenthes khasiana*

The following steps are included in the sterilization process:

1. The seeds of Nepenthes khasiana were collected and washed with distilled water for 4-5 times and washed with tap water for 2-3 times and then the seeds are washed again with distilled water for 2-3 times. These were then kept overnight.
2. Mercuric chloride is measured for 0.1 and 0.5 and then kept in a Petri dish.
3. The seeds of Nepenthes khasiana are washed with distilled water for 2-3 times and soaked with few drops of Dettol for 10-15 minutes and the seeds are washed again in distilled water for 2-3 times.
4. The measured mercuric chloride of 0.1 and 0.5 are then dissolved in 100 ml of distilled water in a conical flask
5. The mixture of 0.5 mercury chloride is poured in a beaker and the seeds are dissolved in that beaker for 5 minutes. Then the seeds are washed in distilled water for 4-5 times. The same process is followed for 0.1 HgCl2.
6. The seeds are washed with 70 % ethanol (70 ml of ethanol and 30ml of distilled water).
7. The next step is to put the seeds in a Petri dish with filter paper placed in the petri dish. The seeds are then spread with a forceps and dried using fan.

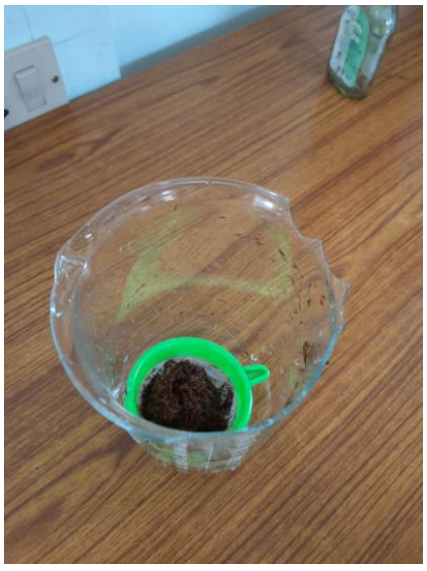

Fig. washed seeds of *Nepenthes khasiana*

INOCULATION OF *Nepenthes khasiana*:

1. This process was proceeded by sterilizing the tools used viz., forceps by flaming them using spirit lamp
2. The seeds were then kept in the bottle where the required medium for its growth is available by spreading the seeds gently across the media
3. The mouth as well as the cap of the bottle were further sterilized by flaming it using the spirit lamp before closing.
4. After all the bottles were filled, they were then taken into the culture room for incubation.

Fig. inoculation of seeds of *Nepenthes khasiana*

Date: 22nd July 2022

Sub-culture of *Cymbidium indicana* and other orchid species & *Nepenthes khasiana*

INTRODUCTION:

In Biology, a subculture is a new cell or microbiological culture made by transferring some or all cells from a previous culture to fresh growth medium. This action is called sub-culturing or passaging the cells. Sub-culture is used to prolong the life and/or expand the number of cells or microorganisms in the culture. In plant tissue culture, Sub-culture is a process in which the plant tissue or explants is first subdivided and transferred into fresh culture medium.

OBJECTIVES:

1. To study plant morphology and physiochemical changes.
2. To sub-culture shoots from the aseptic seedlings, using aseptic technique.
3. To understand callus of various plant species using aseptic technique.

MATERIALS:

- Tube containing MSO medium.
- Tube containing BAP medium.
- Sterile forceps and scalpel with sharp blade
- Cymbidium indicana
- Spatula
- Beaker and bottle jars
- Sterile glass
- Petri dish
- Hot bead sterilizer
- Conical flask sterile distilled water

METHOD:

All the steps are done inside the laminar flow cabinet. Each piece was placed in jars containing MSO and BAP medium. They were then incubated at room temperature and kept inside the culture room.

CONCLUSION:

Subculture is the process by which the tissue or explant is first subdivide, and then transferred into fresh culture medium. It is from a culture of a certain volume into fresh growth medium of equal volume. This allows long-term maintenance of the cell line.

RESULTS:

1. Date: 11th July 2022 and 25th July 2022
   The plants were transferred to mist chamber after hardening.

Fig. plants transferred to mist chamber

2. Date: 12th July 2022,18th July 2022 and 21st July 2022

Fig. Prepared stock for MS media

3. Date: 13th July 2022,15th July 2022 and 21st July 2022
   After the media is prepared, the media was poured into the glass bottles of equal quantity and covered then again covered with the paper. Once done with all the media is kept into the autoclave and kept in laminar air flow.

Fig. Media poured into glass jars

Fig. Media kept in a Laminar air flow

4. Date:19.07.2022 and 20.07.2022
   After inoculation the jar bottles taken to the culture room where they are kept to grow.

Fig. culture room

5. Date: 22nd July 2022

Fig. Plants transfer to a jar containing MSO and BAP medium

DISCUSSION

Plant tissue culture may be used for genetic modification of a plant or simply increase its yield. The cells of the plants can be genetically altered to produce plants with desirable characteristics. This technique utilizes plant's ability to rejuvenate the tissues rapidly. It also helps to conserve plants biodiversity by the production of endangered plants as done in the training where conservation of *Nepenthes khasiana* is well as other endangered orchid species is done. Its advantages and disadvantages are discussed below:

Advantages

- Micropropagation has a number of advantages over traditional plant propagation techniques:
- The main advantage of micropropagation is the production of many plants that are clones of each other.
- Micropropagation can be used to produce disease-free plants.
- It can have an extraordinarily high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this number.
- It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
- It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed cannot be stored (see recalcitrant seeds).
- Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings.
- Some plants with very small seeds, including most orchids, are most reliably grown from seed in sterile culture.
- A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

Disadvantages

- Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include:

- Labor may make up 50%-69% of operating costs.
- A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
- Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
- Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
- Some plants are very difficult to disinfect of fungal organisms.
- The major limitation in the use of micropropagation for many plants is the cost of production; for many plants the use of seeds, which are normally disease free and produced in good numbers, readily produce plants (see orthodox seed) in good numbers at a lower cost. For this reason, many plant breeders do not utilize micropropagation because the cost is prohibitive. Other breeders use it to produce stock plants that are then used for seed multiplication.

Training Report

On

"A study on Plant Tissue Culture"

At

DEPT. OF FOREST & ENVIRONMENT SILVICULTURE DIVISION
TISSUE CULTURE LABORATORY

4 MILE, UPPER SHILLONG, SHILLONG

Submitted by

Suman Basumatary

Department of Biotechnology St.

Edmund's College

Under the Guidance of

Mrs. Badawanshisha Chyne and Mr. Rapki-o Sumer

ACKNOWLEDGEMENT

For the successful completion of the summer training, I would to extend my thanks and gratitude to our head of department Dr. Samrat Adhikari (Dept. of Biotechnology) who gave me this wonderful opportunity to do this training at Department of silviculture and also to the teachers of our department for supporting me direct or indirectly for the fulfillment of this desertion.

I would also like to acknowledge my research guides Ms. Badawanshisha Chyne and Mr. Rapki- o for their continuous guidance during the training.

I would also like to express my gratitude towards the staff in department of silviculture for allowing me to use the laboratory, their lab equipment and also for helping me in many ways.

Suman Basumatary

BSc 5th semester

Dept. of Biotechnology

CONTENT

INTRODUCTION

The training was conducted in Silviculture Division, Tissue Culture Laboratory under the Department of Forest & Environment is situated at 4 ½ mile, Upper Shillong, Shillong. The main objective of the Division is Mapping and Inventorisation of the natural resources of the State for utilization in planning and focusing towards scientific management and exploitation for the ultimate object of sustain yield. The Division also actively engaged in implementing the schemes/works under the Intensification of Forest Management, and also collection of field data like location of plantations, nurseries, beats, ranges, boundary of Reserve Forests, Protected Forests, National Parks and sanctuaries, macro level mapping and preparation of management plan, macro level survey and inventory of Sacred Groves in the different Districts with the main objectives to protect and conserve the Sacred Groves of the State

Micropropagation or tissue culture is the practice of rapidly multiplying plant stock material to produce many progeny plants, using modern plant tissue culture methods.

Micropropagation is used to multiply a wide variety of plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from seedless plants, plants that do not respond well to vegetative reproduction or where micropropagation is the cheaper means of propagating (e.g. Orchids) Cornell University botanist Frederick Campion Steward discovered and pioneered micropropagation and plant tissue culture in the late 1950s and early 1960s.

In short, steps of micropropagation can be divided into 4 stages.

1. Selection of mother plant 2.
   Multiplication
3. Rooting and acclimatizing 4.
   Transfer new plant to soil

Date: 11th and 25th July 2022

Hardening

This is a method in which the tissue culture plants developed in artificial media are habituated to grow in natural environment. Firstly, these plants are taken out from nutrient media and washed thoroughly with water. Then these plants are grown in netted plastic pots filled with liquid nutrient medium and kept in mist chamber for 6 – 8 weeks.

Fig. Plants taken out from nutrient media

9

Fig. Holes poked on plastic cups

This is called Primary hardening. Afterwards the plants are transferred to polybags filled with potting mixture and grown under shaded house for 6 – 8 weeks. This is called Secondary hardening. After secondary hardening the plants are suitable for growing in farmer's fields.

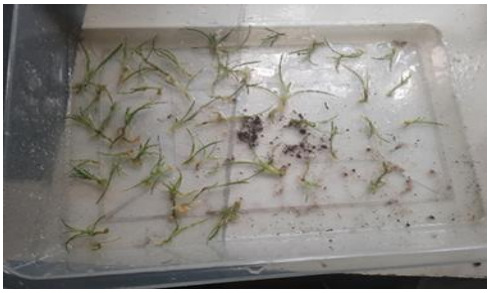

Fig. plants are washed and media is removed

Fig. Plants transferred to potting mixture

Date: 12th July 2022,18th July 2022 and 21st July 2022

Preparation of Plant tissue Culture Stock Solutions

Composition of MS media

Constituent	*Amount(mg/L)*	*For 1L (g/L)*
A. Macro Salts		
NH_4NO_3	1650 x 10 = 16500	16.5
KNO_3	190 x 10 = 1900	19
KH_2PO_4	170 x 10 = 1700	1.7
$MgSO_4.7H_2O$	370 x 10 = 3700	3.7
B. Calcium stock		
$Cacl_2.2H_2O$	440 x 1O = 4400	4.4
C. Micro Salts		
H_3BO_3	6.2 x 100 = 620	0.62
$MnSO_4.7H_2O$	16.9 x 100 =1690	1.69
$ZnSO_4.7H_2O$	8.6 x 100 = 860	0.86
$CuSO_4.5H_2O$	0.025 x 100 = 2.5	0.0025
$Cocl_2.6H_2O$	0.025 x 100 = 2.5	0.0025
KI	0.083 x 100 = 8.3	0.0083
$Na_2MoO_4.2H_2O$	0.25 x 100 = 2.5	0.0025
D. Iron stock		

$FeSO_4.7H_2O$	27.8 x 10 = 278	0.278
Na_2EDTA	37.3 x 10 = 373	0.373
E. Organic stock		
Pyridine-HCl	0.50 x 100 = 50	0.05
Nicotinic Acid	0.50 x 100 = 50	0.05
Thiamine HCl	0.1 x 100 = 10	0.01
Glycine	2 x 100 = 200	0.2

a. Preparation of macro stock (MS-I)

Take 500 ml double distilled water in a 2.0-liter beaker, weigh, add and keep on dissolving each salt sequentially in a descending order as listed above and finally make up the volume to 1000 ml by distilled water.

b. Preparation of calcium stock (MS-II)

Take 500 ml double distilled water in a 2.0-liter beaker, weigh and add $CaCl_2$ .2H2O, dissolve by stirring, and finally make up the volume to 1000 ml by distilled water.

c. Preparation of micro stock (MS-III)

Take 500 ml of double distilled water in a 2.0-liter beaker, weigh, add and keep on dissolving each salt sequentially in descending order as listed above, and finally make up the volume to 1000 ml by distilled water.

d. Preparation of iron stock (MS-IV)

Take 500 ml double distilled water in a 2.0-liter amber colored bottler. Now weigh and add Na_2EDTA and stir; after Na_2EDTA has been dissolved, add gradually $FeSO_4.7H_2O$ while still under mild stirring. This will yield a clear yellow solution.

e. Preparation of organic stock (MS-V)

Take 500 ml double distilled water in a 2.0-liter beaker, keep on adding and dissolving each salts sequentially in descending order as listed above, and finally make up the volume to 1000 ml by adding distilled water.

Fig. Prepared stock for MS media

.

Date: 13th July 2022,15th July 2022 and 21st July 2022

Preparation of MS Media

MATERIALS REQUIRED:

1. Stocks
2. Growth regulators (IAA, NAA, BAP)
3. Marker
4. Glass bottles
5. Sucrose
6. Agar
7. Inositol
8. Large Conical flask
9. Heater or induction
10. Spatula
11. Weighing machine
12. Paper and rubber bands
13. Cloth
14. Micropipette

PROCEDURE ○ MS + 0.1 BAP + 0.1 IAA ○ MS + 0.1 BAP + 0.1NAA ○ MS + 0.1 IAA ○ MS + 0.1 NAA ○ MS + 0.1 IAA O.1 NAA

In a Conical flask adding ○ Macro stock = 100 ml ○ Micro stock 10 ml ○ Cacl2.2H2O = 100ml ○ FeSO4.NaEDTA= 100 ml ○ Organic =10ml ○ Myoinositol = 0.1g ○ Sucrose = 30 g

1. 0.1 BAP and 0.1 IAA was added and the liquid was mixed properly and the PH which must be between 5.6 to 5.8 once done the media was poured into the measuring cylinder then distilled water as added up to 1000ml then the liquid was poured back to the flask then kept it on the induction. Once it heats up Agar about 8 g (and 7.5 g for *Nepenthes)* was added and Shaked vigorously.
2. After that the media was poured into the glass bottles of equal quantity and covered then again covered with the paper.
3. Also following the same procedures for the rest.
4. Once done with all the media is kept into the autoclave.

Fig. Sucrose added in a conical flask.

Fig. pH measure between 5.6 and 5.8

Date:19.07.2022

SURFACE STERILIZATION OF ORCHID SEEDS:

1. The seeds were washed with soap and water.
2. They were then soaked in a solution of 800ml distilled water and 10ml of Dettol in a beaker and were kept for 15-20mins.
3. The seeds were then washed under running water followed by washing it with distilled water.

Fig: Sterilization of Orchid seeds.

. ***INOCULATION OF ORCHID SEEDS***

1. ***1.*** This step is proceeded by further sterilizing the tools used viz., scalpel and forceps by heating them under the spirit lamp.
2. Inoculation of the seeds were done inside a laminar flow where the seeds were dissected into two parts using a scalpel and measures were taken to not contaminate the seeds by not touching the seeds directly with bare hands but instead forceps were used to hold the seed.
3. The inner part of the seed was then used for propagation by carefully spreading them on the required media for its growth by gently tapping the tweezer that is used to pick the seeds on the glass bottle that contains the required medium for the growth of the seed.
4. The cap as well as the bottle was heat sterilized for few seconds before covering the bottle.
5. After all the bottles were filled, they were taken to the culture room where they are kept to grow.

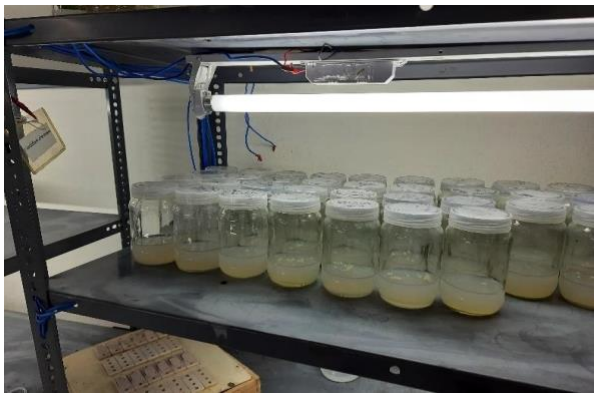

Fig. incubation of orchids

Date:20th July,2022

SURFACE STERILISATION FOR *Nepenthes khasiana*

The following steps are included in the sterilization process:

1. The seeds of Nepenthes khasiana were collected and washed with distilled water for 4-5 times and washed with tap water for 2-3 times and then the seeds are washed again with distilled water for 2-3 times. These were then kept overnight.
2. Mercuric chloride is measured for 0.1 and 0.5 and then kept in a Petri dish.
3. The seeds of Nepenthes khasiana are washed with distilled water for 2-3 times and soaked with few drops of Dettol for 10-15 minutes and the seeds are washed again in distilled water for 2-3 times.
4. The measured mercuric chloride of 0.1 and 0.5 are then dissolved in 100 ml of distilled water in a conical flask

5. The mixture of 0.5 mercury chloride is poured in a beaker and the seeds are dissolved in that beaker for 5 minutes. Then the seeds are washed in distilled water for 4-5 times. The same process is followed for 0.1 HgCl2.
6. The seeds are washed with 70 % ethanol (70 ml of ethanol and 30ml of distilled water).
7. The next step is to put the seeds in a Petri dish with filter paper placed in the petri dish. The seeds are then spread with a forceps and dried using fan.

Fig. washed seeds of *Nepenthes khasiana*

INOCULATION OF *Nepenthes khasiana*:

1. This process was proceeded by sterilizing the tools used viz., forceps by flaming them using spirit lamp
2. The seeds were then kept in the bottle where the required medium for its growth is available by spreading the seeds gently across the media
3. The mouth as well as the cap of the bottle were further sterilized by flaming it using the spirit lamp before closing.
4. After all the bottles were filled, they were then taken into the culture room for incubation.

Fig. inoculation of seeds of *Nepenthes khasiana*

Date: 22nd July 2022

Sub-culture of *Cymbidium indicana* and other orchid species & *Nepenthes khasiana*

INTRODUCTION:

In Biology, a subculture is a new cell or microbiological culture made by transferring some or all cells from a previous culture to fresh growth medium. This action is called sub-culturing or passaging the cells. Sub-culture is used to prolong the life and/or expand the number of cells or microorganisms in the culture. In plant tissue culture, Sub-culture is a process in which the plant tissue or explants is first subdivided and transferred into fresh culture medium.

OBJECTIVES:

1. To study plant morphology and physiochemical changes.
2. To sub-culture shoots from the aseptic seedlings, using aseptic technique.
3. To understand callus of various plant species using aseptic technique.

MATERIALS:

- Tube containing MSO medium. ○ Tube containing BAP medium.
- Sterile forceps and scalpel with sharp blade
- Cymbidium indicana ○ Spatula
- Beaker and bottle jars
- Sterile glass ○ Petri dish ○ Hot bead sterilizer
- Conical flask sterile distilled water

METHOD:

All the steps are done inside the laminar flow cabinet. Each piece was placed in jars containing MSO and BAP medium. They were then incubated at room temperature and kept inside the culture room.

CONCLUSION:

Subculture is the process by which the tissue or explant is first subdivide, and then transferred into fresh culture medium. It is from a culture of a certain volume into fresh growth medium of equal volume. This allows long-term maintenance of the cell line.

RESULTS:

1.Date: 11th July 2022 and 25th July 2022

The plants were transferred to mist chamber after hardening.

Fig. plants transferred to mist chamber

2.Date: 12th July 2022,18th July 2022 and 21st July 2022

Fig. Prepared stock for MS media

3.Date: 13th July 2022,15th July 2022 and 21st July 2022

After the media is prepared, the media was poured into the glass bottles of equal quantity and covered then again covered with the paper. Once done with all the media is kept into the autoclave and kept in laminar air flow.

Fig. Media poured into glass jars

Fig. Media kept in a Laminar air flow

4.Date:19.07.2022 and 20.07.2022

After inoculation the jar bottles taken to the culture room where they are kept to grow.

Fig. culture room

5.Date: 22nd July 2022

Fig. Plants transfer to a jar containing MSO and BAP medium

DISCUSSION

Plant tissue culture may be used for genetic modification of a plant or simply increase its yield. The cells of the plants can be genetically altered to produce plants with desirable characteristics. This technique utilizes plant's ability to rejuvenate the tissues rapidly. It also helps to conserve plants biodiversity by the production of endangered plants as done in the training where conservation of *Nepenthes khasiana* is well as other endangered orchid species is done. Its advantages and disadvantages are discussed below:

Advantages

- Micropropagation has a number of advantages over traditional plant propagation techniques:
- The main advantage of micropropagation is the production of many plants that are clones of each other.
- Micropropagation can be used to produce disease-free plants.

- It can have an extraordinarily high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this number.
- It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
- It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed cannot be stored (see recalcitrant seeds).
- Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings.
- Some plants with very small seeds, including most orchids, are most reliably grown from seed in sterile culture.
- A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

Disadvantages

- Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include:
- Labor may make up 50%-69% of operating costs.
- A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
- Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
- Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
- Some plants are very difficult to disinfect of fungal organisms.

- The major limitation in the use of micropropagation for many plants is the cost of production; for many plants the use of seeds, which are normally disease free and produced in good numbers, readily produce plants (see orthodox seed) in good numbers at a lower cost. For this reason, many plant breeders do not utilize micropropagation because the cost is prohibitive. Other breeders use it to produce stock plants that are then used for seed multiplication.

TRAINING REPORT

ON

SUMMER TRAINING

AT

DEPT. OF FOREST &ENVIRONMENT

SILVICULTURE DIVISION

TISSUE CULTURE LABORATORY

UPPER SHILLONG

Submitted By:

Pynbianglang Dkhar

Department of Biotechnology

St. Edmund's College

Under Supervision of Mr. Rapki-o sumer & Mrs. Badaiahunlang Chyne

CONTENTS

ACKNOWLEDGEMENT

I take this opportunity to express my gratitude and appreciation to all those who gave me the possibility on completing this training. Firstly, I would thank God for giving me good health and strength and all the unseen blessings He has bestowed upon me. I would also like to thank my parents and family for their support and encouragement.

Secondly, I would like to thank our Head of Department, Department of Biotechnology, Dr. Samrat Adhikari and all the staff for giving us this opportunity to be able to learn new things.

Also, I extend special thanks of gratitude to Mr. Rapki-o Sumer and Mrs. Badawanshisha Chyne, the associates at the Dept. of Silviculture who has patiently trained and guided us throughout the training and also the staff and workers for their kindness and help that has made this learning experience a great one.

Lastly, I would like to thank all my friends who has been part of the training for being co-operative their teamwork that has made the completion of this training a successful one.

INTRODUCTION

The Silviculture Division, Tissue Culture Laboratory under the Department of Forest & Environment is situated at 4 ½ mile,Upper Shillong,Shillong .The main objective of the Division are mapping and inventorisation of the natural resources of the State for utilization in planning and focusing towards scientific management and exploitation for the ultimate object of sustain yield. The Division also actively engaged in implementing the schemes works under the Intensification of Forest Management, and also collection of field data like location of plantations, nurseries, beats, ranges, boundary of Reserve Forests, Protected Forests, National Parks and sanctuaries, macro level mapping and preparation of management plan, macro level survey and inventory of Sacred Groves in the different Districts with the main objectives to protect and conserve the Sacred Groves of the State.

- The activities of the Silviculture Division are given below:
- Creation of Seed Orchards and identification of Superior/Plus Tree species.
- Standardizing Nursery Practices and Propagation through tissue culture techniques of Rare, Endangered and Critically Endangered species and restock in the natural habitat.
- Protection and Conservation of Rare, Endangered & Endemic Plant Species through in- situ Conservation and protection from natural and biotic pressure.
- Protection and Conservation of Rare, Endangered & Endemic Plant Species and medicinal Plants through ex- situ conservation viz. Germplasm bank, creation and maintenance of herbal garden.
- Conservation & Developing protocols of important tree and Bamboo species of the State.
- Micropropagation and development of tissue culture protocols of Rare, Endangered & Endemic Plant Species including Orchids and Medicinal Plant, *Nepenthes khasiana* (Pitcher plant) and other plants.

Tissue Culture: Micropropagation or tissue culture is the practice of rapidly multiplying plant stock material to produce many progeny plants, using modern plant tissue culture methods. Micropropagation is used to multiply a wide variety of plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from seedless plants, plants that do not respond well to vegetative reproduction or where micropropagation is the cheaper means of propagating (e.g. Orchids) Cornell University botanist Frederick Campion Steward discovered and pioneered micropropagation and plant tissue culture in the late 1950s and early 1960s. In short, steps of micropropagation can be divided into 4 stages:

1. Selection of mother plant
2. Multiplication
3. Rooting and acclimatization
4. Transfer new plant to soil

Date- 11th & 25th July, 2022

HARDENING

This is a method in which the tissue culture plants developed in artificial media are habituated to grow in natural environment. Firstly, these plants are taken out from nutrient media and washed thoroughly with water. Then these plants are grown in netted plastic pots filled with liquid nutrient medium and kept in mist chamber for 6 – 8 weeks.

Fig 1: Plants taken out from the nutrient media

Fig 2: Holes poked on the cups

This is called Primary hardening. Afterwards the plants are transferred to polybags filled with potting mixture and grown under shaded house for 6 – 8 weeks. This is called Secondary hardening. After secondary hardening the plants are suitable for growing in farmer's fields.

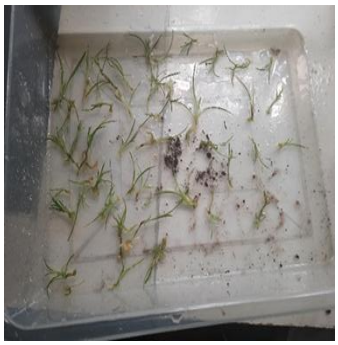

Fig 3: Plants are washed to remove media

Fig 4: Transferred to potting mixture

Fig 5: Plants transferred to the mist chamber

Hardening is the weaning of plants from the protective, sterile, humid climate through a transitional phase to enable them to survive the harsh ,dry climate.(Thiart,2004,Baguma et al.,2010).This phase is done to minimize water loss, facilitate regaining of normal stomatal functioning to regulate transpiration, facilitate rooting. These minimize rapid wilting during transplantation and enhance survival rates at establishment(Conner and Thomas 1982).

Date- 16th & 18th July, 2022

PREPARATION OF PLANT TISSUE STOCK SOLUTIONS

Composition of MS media

Constituent	*Amount(mg/L)*	*For 1L (g/L)*
A. Macro Salts		

NH_4NO_3	1650 x 10 = 16500	16.5
KNO_3	190 x 10 = 1900	19
KH_2PO_4	170 x 10 = 1700	1.7
$MgSO_4.7H_2O$	370 x 10 = 3700	3.7
B. Calcium stock		
CaCl2.2H2O	440 x 1O = 4400	4.4
C. Micro Salts		
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CoCl2.6H2O	0.025 x 100 = 2.5	0.0025
KI	0.083 x 100 = 8.3	0.0083
Na2MoO4.2H2O	0.25 x 100 = 2.5	0.0025
D. Iron stock		
FeSO4.7H2O	27.8 x 10 = 278	0.278
Na2EDTA	37.3 x 10 = 373	0.373
E. Organic stock		
Pyridine-HCl	0.50 x 100 = 50	0.05
Nicotinic Acid	0.50 x 100 = 50	0.05
Thiamine HCl	0.1 x 100 = 10	0.01

Glycine	2 x 100 = 200	0.2

A. Preparation of macro stock (MS-I)

500ml double distilled water is taken in a 2.0-liter beaker, weighed, each salt is sequentially added and kept on dissolving in a descending order as listed above and finally the volume is made upto 1000ml by distilled water.

B. Preparation of calcium stock (MS-II)

500ml double distilled water is taken in a 2.0-liter beaker, weighed and $CaCl_2$ .2H2O is added, dissolved by stirring, and finally the volume is made up to 1000ml by distilled water.

C. Preparation of micro stock (MS-III)

500ml of double distilled water is taken in a 2.0-liter beaker, weighed, each salt is sequentially added and kept on dissolving in a descending order as listed above, and finally the volume is made up to 1000 ml by distilled water.

D. Preparation of iron stock (MS-IV)

500ml double distilled water is taken in a 2.0-liter amber colored bottle. Then, Na_2EDTA is weighed and added and stir; after Na_2EDTA has been dissolved, $FeSO_4.7H_2O$ is added gradually while still under mild stirring. This will yield a clear yellow solution.

E. Preparation of organic stock (MS-V)

500ml double distilled water is taken in a 2.0-liter beaker, keep on adding and dissolving each salts sequentially in descending order as listed above, and finally the volume is made up to 1000ml by adding distilled water.

Fig 6: Prepared stock for MS media

Date- 13th, 15th, 18th & 21st July, 2022

PREPARATION OF MS MEDIA FOR ORCHIDS & *Nepenthes khasiana*

MATERIALS REQUIRED:

1. Stocks
2. Growth regulators (IAA, NAA, BAP)
3. Marker
4. Glass bottles
5. Sucrose
6. Agar
7. Inositol
8. Large Conical flask
9. Heater or induction
10. Spatula
11. Weighing machine
12. Paper and rubber bands
13. Cloth
14. Micropipette

PROCEDURE:

- MS + 0.1 BAP + 0.1 IAA
- MS + 0.1 BAP + 0.1NAA
- MS + 0.1 IAA
- MS + 0.1 NAA
- MS + 0.1 IAA O.1 NAA

In a Conical flask adding

- Macro stock = 100 ml
- Micro stock 10 ml
- CaCl2.2H2O = 100ml
- FeSO4.NaEDTA= 100 ml
- Organic =10ml
- Myo-inositol = 0.1g
- Sucrose = 30 g

1. 0.1 BAP and 0.1 IAA is added and the liquid is mixed properly and the pH must be between 5.6 to 5.8. Once done the media was poured into the measuring cylinder then distilled water is added up to 1000ml, then the liquid is poured back to the flask then kept it on the induction. Once it heats up Agar about 8 g (and 7.5 g for *Nepenthes)* is added and shaken vigorously.
2. After that the media is poured into the glass bottles of equal quantity and closed and then covered with the paper.
3. Also following the same procedures for the rest.
4. Once done with all the media is kept into the autoclave.

Fig 7: Measuring the pH

Fig 8: Adding sucrose to mixture

Fig 9: Media poured into glass jars

Fig 10: Media kept in Laminar Flow

Date-19th July, 2022

SURFACE STERILIZATION FOR ORCHID SEEDS

- The seeds were washed with soap and water.
- They were then soaked in a solution of 800ml distilled water and 10ml of Dettol in a beaker and was kept for 15-20mins.
- The seeds were then washed under running water followed by washing it with distilled water.

Fig 11: Sterilization of Orchid seeds

Inoculation of Orchid seeds

- This step is proceeded by further sterilizing the tools used viz.,scalpel & forceps by heating them under the spirit lamp.
- Inoculation of the seeds were done inside the laminar flow where the seeds are dissected into two parts using a scalpel and measures are taken to not contaminate the seeds by not touching the seeds directly with bare hands but instead forceps are used to hold the seed.
- The inner part of the seed was then used for propagation by carefully spreading them on the required media for its growth by gently tapping the tweezer that is used to pick the seeds on the glass bottle that contains the required medium for the growth of the seed.
- The cap as well as the bottle was heat sterilized for few seconds before covering the bottle.
- After all the bottles were filled they were taken to the culture room where they are kept to grow.

Fig 12: Inoculation of orchid seeds inside the Laminar Flow

Date- 17th July, 2022

SURFACE STERILIZATION FOR *Nepenthes khasiana*

The following steps are included in the sterilization process:

- Mercuric chloride is measured for 0.1g and 0.5g and then kept in a Petri dish.
- The seeds of *Nepenthes khasiana* are washed with distilled water for 2-3 times and soaked with few drops of Dettol for 10- 15 minutes and the seeds are washed again in distilled water for 2-3 times .These were then kept overnight.
- The measured mercuric chloride of 0.1 and 0.5 are then dissolved in 100 ml of distilled water in a conical flask.
- The mixture of 0.5 mercury chloride is poured in a beaker and the seeds are dissolved in that beaker for 5 minutes. Then the seeds are washed in distilled water for 4-5 times. The same process is followed for 0.1 HgCl2.
- The seeds are washed with 70 % ethanol (70 ml of ethanol and 30ml of distilled water.
- The next step is to put the seeds in a Petri dish with filter paper placed in the petri dish. The seeds are then spread with a forcep and dried using fan.

Inoculation of *Nepenthes khasiana:*

- This process was proceeded by sterilizing the tools used viz., forceps by flaming them using spirit lamp.
- The seeds were then kept in the bottle where the required medium for its growth is available by spreading the seeds gently across the media.
- The mouth as well as the cap of the bottle were further sterilized by flaming it using the spirit lamp before closing.
- After all the bottles were filled,they were then taken into the culture room.

Fig. inoculation of seeds of *Nepenthes khasiana*

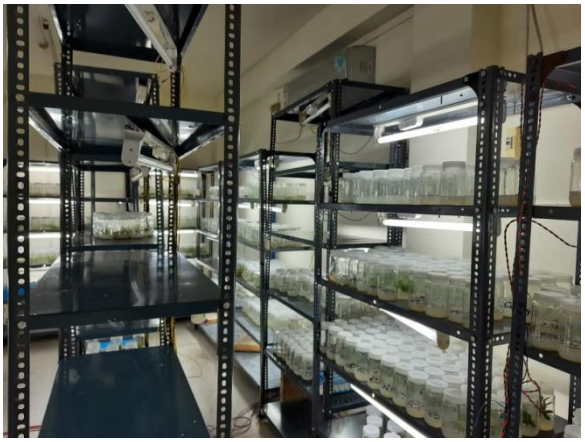

Fig 13: Culture room

.Date- 22nd July, 2022
SUB-CULTURE OF *Cymbidium indicana* AND OTHER ORCHID SPECIES & *Nepenthes khasiana*

In Biology, a subculture is a new cell or microbiological culture made by transferring some or all cells from a previous culture to fresh growth medium. This action is called sub-culturing or passaging the cells. Sub-culture is used to prolong the life and/or expand the number of cells or microorganisms in the culture. In plant tissue culture, Sub-culture is a process in which the plant tissue or explants is first subdivided and transferred into fresh culture medium.

OBJECTIVES:

1. To study plant morphology and physiochemical changes.
2. To sub-culture shoots from the aseptic seedlings, using aseptic technique.
3. To understand callus of various plant species using aseptic technique.

MATERIALS:

- Tube containing MSO medium.
- Tube containing BAP medium.
- Sterile forceps and scalpel with sharp blade

- *Cymbidium indicana(*also *Nepenthes khasiana)*
- Spatula
- Beaker and bottle jars
- Sterile glass
- Petri dish
- Hot bead sterilizer
- Conical flask sterile distilled water

METHOD:

All the steps are done inside the laminar flow cabinet. Each piece was placed in jars containing MSO and BAP medium. They were then incubated at room temperature and kept inside the culture room.

RESULT:

Fig 14: Plants transferred to jars containing MSA and BAP medium

CONCLUSION:

Subculture is the process by which the tissue or explant is first subdivide, and then transferred into fresh culture medium. It is from a culture of a certain volume into fresh growth medium of equal volume. This allows long-term maintenance of the cell line.

DISCUSSION:

Advantages

Micropropagation has a number of advantages over traditional plant propagation techniques:

The main advantage of micropropagation is the production of many plants that are clones of each other.

•Micropropagation can be used to produce disease-free plants.

•It can have an extraordinarily high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this number.
•It is the only viable method of regenerating genetically modified cells or cells after protoplast fusion.
•It is useful in multiplying plants which produce seeds in uneconomical amounts, or when plants are sterile and do not produce viable seeds or when seed cannot be stored (see recalcitrant seeds).
•Micropropagation often produces more robust plants, leading to accelerated growth compared to similar plants produced by conventional methods - like seeds or cuttings.
•Some plants with very small seeds, including most orchids, are most reliably grown from seed in sterile culture.
•A greater number of plants can be produced per square meter and the propagules can be stored longer and in a smaller area.

Disadvantages

Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include:
•Labor may make up 50%-69% of operating costs.
•A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
•An infected plant sample can produce infected progeny. This is uncommon as the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.
•Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the plants produce secondary metabolic chemicals that stunt or kill the explant.
•Sometimes plants or cultivars do not come true to type after being tissue cultured. This is often dependent on the type of explant material utilized during the initiation phase or the result of the age of the cell or propagule line.
•Some plants are very difficult to disinfect of fungal organisms.
•The major limitation in the use of micropropagation for many plants is the cost of production; for many plants the use of seeds, which are normally disease free and produced in good numbers, readily produce plants (see orthodox seed) in good numbers at a lower cost. For this reason, many plant breeders do not utilize micropropagation because the cost is prohibitive. Other breeders use it to produce stock plants that are then used for seed multiplication.

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Summer training report

ICAR RESEARCH COMPLEX FOR NEH REGION

DIVISION OF ANIMAL HEALTH AND FISHERIES SCIENCE

Umiam, Meghalaya

Submitted By: Rohini Gautam

BSc. Biotechnology

St. Edmund's College, Shillong-4

Acknowledgement

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Finally, I would express my gratitude to our principal pf St. Edmund's College Dr. Sylvanus Lamare who gave the Golden opportunity to do this Summer Training Programme.

Contents

Introduction

A summer training programme was conducted for 15 days from 13 July 2022 till 27 July 2022 by St Edmunds College, Shillong for BSc Biotechnology students in Indian Council of Agricultural Research, Umiam under the division of animal health.

This programme was basically concerned in providing the students a wider picture of their interests in the various fields of science. The training helped us to improve the practical skills and make critical analysis and approach the innovative techniques while performing an experiment. We were given an exposure in fields such as fisheries, agriculture, biochemistry, botany and were introduced to different machines that were required in experiments.

In these fifteen days, this training served us a lot of purposes. It has helped us to enhance our knowledge and to also know how biotechnology has helped to ease up the techniques in the different fields of science.

Date: 13 July 2021

Resource Person: Dr K. Puro

Polymerase Chain Reaction (PCR)

Polymerase chain reaction or pcr is an in-vitro method that is designed to permit selective amplification of a specific target DNA sequence within a heterogeneous collection of DNA sequences (e.g., total genomic DNA or a complex c-DNA population).

- **Principle of PCR**

PCR methods are based on thermal cycling that exposes the reactants to repeated cycles of heating and cooling to permit different temperature dependent reactions I.e., DNA melting, primer annealing and polymerase driven DNA replication. PCR employs three main reagents, primers (two short single stranded forward and reverse DNA fragments), DNA polymerase (Taq polymerase) and a chemical component known as master mix (consists of magnesium chloride, Buffer, and deoxynucleotide triphosphate or dNTPs) are required.

Following are the three main stages

1. Denaturing – when the double-stranded template DNA is heated to separate it into two single strands.
2. Annealing – when the temperature is lowered to enable the DNA primers to attach to the template DNA.
3. Extending – when the temperature is raised and the new strand of DNA is made by the Taq polymerase enzyme.

As PCR progresses, the DNA generated is itself used as a template for replication, thus setting in motion a chain reaction in which the original DNA fragment is exponentially amplified.

Antibacterial Susceptibility Test

Antibacterial Susceptibility Testing or AST is a procedure used to determine on which antibiotics a specific organism or group of organisms are susceptible to.

The standard procedure for assessing antibacterial activity is the disc diffusion test**.** In this method the bacteria and the antibody are inoculated in a media inside the petri dish and are kept in incubation for 24 hours. Zone of equivalence was observed. The Zone of equivalence is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. It is used to measure the susceptibility of the bacteria towards the antibiotic. . There were two types of conditions; one with a larger zone of equivalence around the antibody which gave an indication that the bacterial colonies are susceptible to that antibody. The other condition showed a smaller zone of equivalence around the antibody which gave an indication that the bacterial colonies are resistant to that antibody. The bacteria were a gram positive called *Klebsiella.*

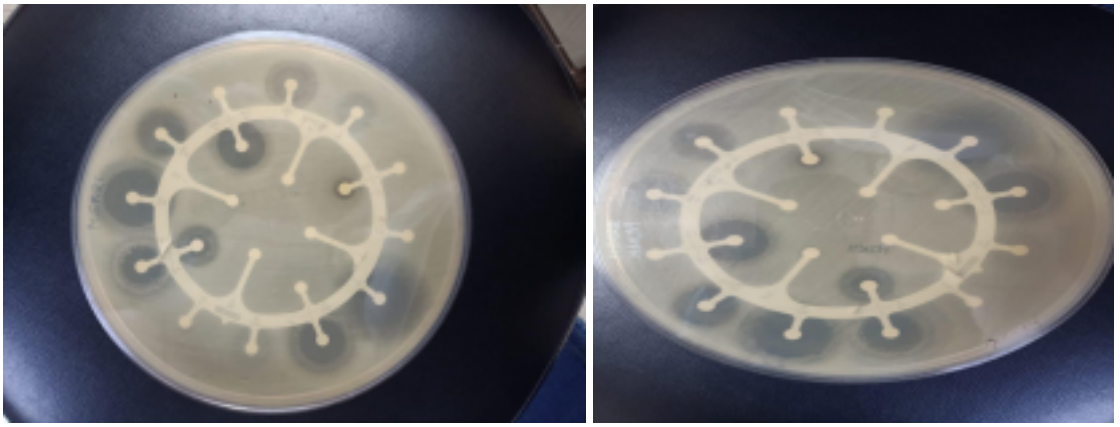


Fig: Antibacterial Susceptibility Test of the bacteria *Klebsiella*

Inoculation of *Clostridium perfringens*

Clostridium perfringens is a gram-positive bacterium which can be found in the intestine of the chicken. The samples were taken from the intestine of different chickens and were inoculated in the RCM (Robertson's Cooked Meat medium) media and was kept under incubation for 24 hours.

Observation: There was a change in colour and density of the sample. The reaction is

known as proteolytic reaction.

Date: 14 July 2022

Resource Person: Dr. S Das

Agarose gel electrophoresis

Agarose gel electrophoresis is a form of electrophoresis used for the separation of nucleic acid (RNA) fragments based on their size. Negatively charged RNA migrated through the pores of an agarose gel towards the positively charged end of the gel when an electrical current was applied and the smaller fragments migrate faster. The resulting bands were then visualised using ultraviolet (UV) light.

Avian pox

Fowl pox is a highly contagious viral infection in poultry that causes painful sores on a chicken's skin. It is also referred to as Avian pox. Some samples were collected from the skin of the chicken. The symptoms showed relatable characteristics of avian pox. Hence the diagnosis further led to the detection of whether the bird suffered through the assumed disease or not. Before starting the process, some information was collected from the ncbi site like sequencing of forward and reverse primers and the number of base pairs of the avian pox which was 578bp. After collecting the data, the identification of the disease was done by the following steps.

- Trituration with sand and 1 x PBS buffer
- Freezing and thawing (freeze the sample for 24 hours)
- DNA extraction
- PCR
- Gel electrophoresis

Biosafety level 3 (BSL-3)

Biosafety level – 3 is totally an enclosed, ventilated cabinet with leak-tight construction and attached rubber gloves for performing operations in the cabinet. Class III biosafety cabinets are also called glove boxes. The cabinet has a transfer chamber that allows for sterilising materials before they leave the glove box. The cabinet is maintained under negative pressure and supply air is drawn in through HEPA filters. The exhaust air is treated with either double HEPA filtration or HEPA filtration and incineration. Class III cabinets are safe for work requiring Biosafety Level 1, 2, 3 or 4 containments.

BSL-3 laboratories are used to study infectious agents or toxins that may be transmitted through the air and cause potentially lethal infections.

Fig: BSL Laminar Flow

Observation of the Mycobacterium sp.

Mycobacterium tuberculosis is a slow-growing bacterium that is the etiological agent of tuberculosis which were cultured in a slant media. It causes some lethal diseases in both humans and animals. Some examples of *Mycobacteria chelonae* and Mycobacterium *bovis*

Date: 15 July 2022

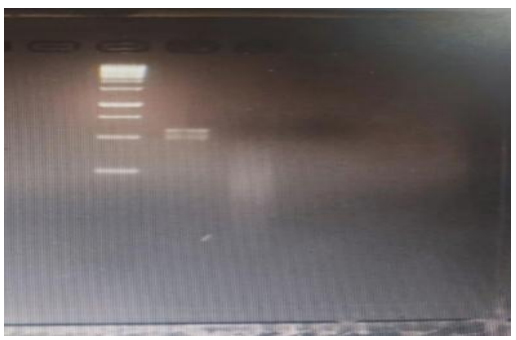
Resource person: Dr Milton

Detection of avian pox

After thawing, the DNA was extracted and was run into the PCR. Then passing through the process of PCR the samples were taken for electrophoresis techniques. The first well was loaded with the sample, the second well was loaded with the positive control and the third well was loaded with the non-template control and the last well was loaded with a 1kb ladder.

According to ncbi the base pair for avian pox is 578 that is the DNA band must be seen in between 500-750 bp

Observation: The band was observed in 500-750 bp lane of positive control but not in the lane of the sample which gave the result that the chicken was avian negative. The picture below shows the result of the observation.



Date: 16 July 2022

Resource Person: Dr. Sen

Innate and adaptive immunity

- **Innate immunity:** The innate immune system is the first part of the body to detect invaders such as viruses, bacteria, parasites and toxins, or to sense wounds or trauma. Upon detection of these agents or events, the innate immune system activates cells to attack and destroy the outsider, or to initiate repair, while also informing and modulating the *adaptive* immune response that follows this first line of defence.

- **Adaptive immunity:** Adaptive immune cells are the second and specific line of defence, and they are called to action by the innate immune system. After recognizing the invader, the cells can multiply and combat it, leading to recovery from disease and protection against its return.

Toll-like Receptors (TLRs)

Toll-like receptors (TLRs) play crucial roles in the innate immune system by recognizing pathogen-associated molecular patterns derived from various microbes. TLRs signal through the recruitment of specific adaptor molecules, leading to activation of the transcription factors NF-κB and IRFs, which dictate the outcome of innate immune responses.

Neutralising and non-neutralizing antibodies

- **Neutralising antibody:** A neutralising antibody (NAb) is an antibody that is responsible for defending cells from pathogens, which are organisms that cause disease. They are produced naturally by the body as part of its immune response, and their production

is triggered by both infections and vaccinations against infections.

- **Non neutralising antibody:** Non neutralising antibodies bind specifically to the pathogens but do not interfere with their infectivity as they do not bind to the right region.

Date: 18 July 2022

Resource Person: Dr. Sanjukta

Nanotechnology

Nanotechnology is a discipline of science that involves the engineering of materials and objects at an extremely small scale - just larger than that of atoms. Nanotechnology is used across a range of industries to drive improvements that are possible only because of the special material interactions and operations that occur at the small size level with which nanotechnology is concerned.
Nanoparticles can be classified into different types according to size, morphology and physical and chemical properties. Some of them are carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles.

Applications

- Nanoparticles are used in the food of the animals to assure the antimicrobial activity which can provide resistance to any abnormalities in animals.
- Nanotechnology can provide rapid and sensitive detection of cancer related molecules.
- It has a range of potential applications for animal production systems, including new tools to aid animal breeding, targeted disease treatment delivery systems, new materials for pathogen detection, and identity preservation systems.
- It is also used in cosmetics and in ornaments as well.

Conclusion

Nanoscience and nanotechnology involve the ability to see and to control individual atoms and molecules. Today's scientists and engineers are finding a wide variety of ways

to deliberately make materials at the nanoscale to take advantage of their enhanced properties such as higher strength, lighter weight, increased control of light spectrum, and greater chemical reactivity than their larger-scale counterparts.

Streaking method of bacterial culture

In microbiology, streaking is a technique used to isolate a pure strain from a single species of microorganism, often bacteria.

There are different streaking methods of bacterial culture, one of which is a quadrant method which was performed in a laminar flow. The quadrant streaking method's principle involves inoculation of a little inoculum on successive quadrants of the solid agar surface. The number of organisms in the inoculum decreases by sequential streaking. Eventually, the inoculum was diluted to a point where a single bacterial cell growth occurs after every few millimetres on the agar surface. It uses a sterilised inoculating loop to make striations over the solid culture medium.

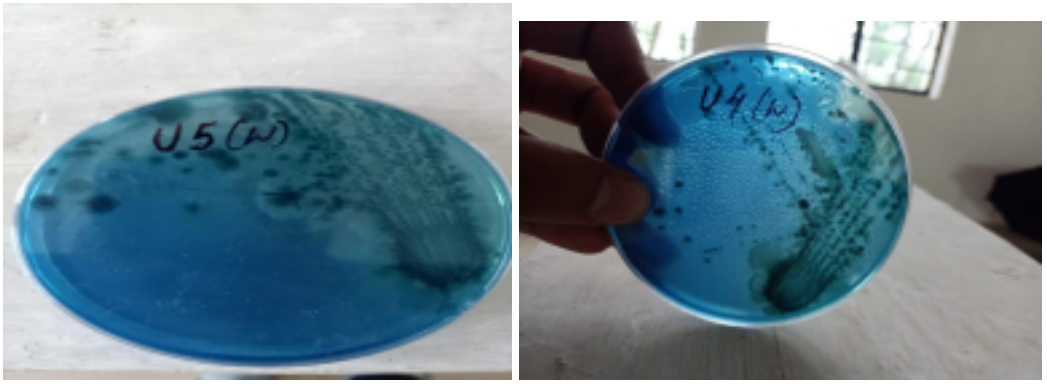


Fig: Bacterial culture by streaking method

Date: 19 July 2022

Resource Person: Dr. Katiyar

Semen Analysis

Artificial insemination

Artificial insemination or A.I is the insertion and delivery of semen from boar into the reproductive canal of a female pig (gilt or sow) during the oestrus period. During insemination, hydraulic pressure turns the sleeve inside out. The sleeve threads through the cervix and deposits semen directly into the uterus. The rods are available in either gilt or sow models. Squeeze and rods are locked into the cervix like any foam-tipped rod.

At first the semen is collected from the Boar which must be kept in a thermostable bottle, as the sperm cells (37°C) are sensitive to the temperature and may die if the temperature is not maintained. So, to maintain the temperature of the sperm, it is kept in water bath by providing the temperature between 35-37°C. The semen is then diluted with a preheated extender to preserve sperm by stabilising its properties including sperm morphology, motility rate which must be 75% and viability.

Before performing artificial insemination, several other conditions must be kept in mind i.e. the timing of the insemination. The sow or gilt must be matured enough which means it should undergo its puberty stage which is 6 to 8 months after the birth, it acquires its sexual maturity for about 6-12 months.

Once the gilt or sow is sexually matured the process of artificial insemination can begin. Females should only be bred when in standing heat i.e. they need to be inseminated before ovulation which occurs at the end of the oestrous cycle; however, it is very difficult to

determine when they will ovulate so hence, they must be inseminated twice. The first insemination must be done 12 hours after the first detection of oestrus and the second one should follow 12 hours after the first. Observation of physiological and behavioural signs are the most common method used for pregnancy diagnosis. Swelling of the vulva portion, mucous discharge, restlessness, vulva reddening and loss of appetite. The average pregnancy duration is 114 days which is 3 months3 week and 3 days.

Riding test or Back pressure test is the technique used for insemination in pigs. In this test a female pig will stand in response to back pressure without the presence of the boar. However, many gilts and sows will not exhibit the standing reflex when pressure is applied to their back unless a boar is present.

Date: 20 July 2022

Resource Person: Dr. C Debnath

Fishery Science

Fisheries science is the study of freshwater, marine and/or estuarine aquatic systems that involve humans. A fishery is any body of water with which humans interact, such as in the form of fishing or swimming. A body of water that does not involve human interaction in one form or another is not a fishery. Different types of artificial ponds are created for culturing the different types of fish in which accordingly few parameters are maintained.

● Fish breeding

Fish breeding means the breeding, nursing and transfer of fish from one water body into another, also catching and keeping the reproducers for the recovery, maintenance and increase of fisheries resources. The picture below shows the eggs of

● Fish hatchery

A fish hatchery is a facility designed to raise fish. It provides an optimum environment for fish eggs to develop and hatch by maintaining proper water temperature and oxygen levels, and providing adequate food supplies and safety from predators. The eggs and sperm

are collected in a common trough that feeds into a bucket. Water is added to the eggs and sperm to induce fertilisation. The excess sperm, ovarian fluid, and blood are rinsed away. The fertilised eggs are gently poured into an incubator tray. The picture below shows the eggs of the fish.

Fig: Eggs of the fish

- **Preparation of fish feed**

The food of the fish can be prepared by Pearson Square Method. The Pearson Square is a tool that can be used to calculate the portion of two feeds needed to meet the protein energy requirements of an animal. For this tool to work, one of the feeds used must be higher in protein or carbohydrates than the desired level and the other feed must be below the desired level. The picture below shows the prepared fish feed before drying it.

Fig: Preparation of fish food

- **Fish dissection**

Dissection of fish aims to help students become familiar with the internal organs of a fish. The picture below shows the dissection of a fish known as **Common carp** or ***Cyprinus Carpio.*** Common Carp is a large, deep-bodied fish, varying in colour from silver to olive-green, brass or grey on the back and sides. Its belly is yellowish and the lower fins are

orange-red. It has a single dorsal spine and its cheeks and gill covers are partially scaled.

Date: 21 July 2022

Resource Person: Dr. C Aochen

Estimation of amino acid

Lysine is the limited amino acid in the cereal grains. Assessment of lysine in cereal grains for nutritional quality is hence one of the procedures adopted for screening varieties. Though an ideal simple method for direct estimation of lysine is yet to be found, the given procedure is sufficient for routine screening.

Principle

The protein in the grain sample is hydrolysed with a proteolytic enzyme, papain. The alpha-amino group of the derived amino acids are made to form a complex with copper. The e -amino group of lysine which does not couple with copper is made to form e -dinitro pyridyl derivative of lysine with 2-chloro-3, 5-dinitropyridine. The excess pyridine is removed with ethyl acetate and the colour of the e-dinitro pyridyl derivative is read at 390nm.

Date: 22 July 2022

Resource Person: Dr. A. Ratan

Plant pathology

Plant Pathology is the science of studying plant diseases that renders the disease-management answers to the farmers.

- ***Fusarium spp.***

  Fusarium spp. is a pathogenic fungus causing stem rot disease on Dragon Fruits which is one of the main fungal agents causing pathogenic disease. It produces colonies that are white and cottony. Initial symptoms show the appearance of brown lesions and gradually the disease raised up to the upper stem. As the disease became more severe, the lesions enlarged and turned soft and watery.

Slide Preparation

An infinitesimal small amount of white and cottony colonies was taken onto the slide. It was then stained with water (could also be stained with methyl blue dye) followed by covering it with the help of a cover slip and was viewed under microscope.

Observation: Under the microscope mycelium with conidia was observed.

Isolation of Fusarium species from dragon fruit

- At first, the mycelium sheath was incubated in potato dextrose broth
- In the laminar flow the mycelia were harvested
- The mycelia sheath was grounded in a mortar and pestle. Then trituration, freezing and thawing, DNA extraction, PCR and gel electrophoresis was followed.

Date: 23rd July 2022

Recourse Person: Dr Sen

Cell culture

Cell culture is a method used to cultivate, propagate and grow a large number of cells in a dish. The cells can be of a mixed, heterogeneous origin with different cell types growing, or they can be a singular cell type, sometimes clonal in origin. Cell culture allows one to grow cells outside of their natural environment and control the conditions in which they grow.

Using the tissue culture technique, the infected poultry bird's cells were grown in a flask using various broth media and then these cells were infected with the virus. The inclusion bodies were then observed under the microscope. After the observation, it proved that the cells were affected by the virus. The affected cells were subcultured, known as passaging. Passaging is the procedure of harvesting cells from a culture, transferring the cells to one or more culture vessels with fresh growth medium, and using those cells to start new cultures.

After passing, cell detachment is important and the process is known as trypsinization. Trypsinization is the process of cell dissociation using trypsin, a proteolytic enzyme which breaks down proteins, to dissociate adherent cells from the vessel in which they are being cultured.

- **Primary cell culture**: Primary cell culture is the first line of cells directly taken from the target organism. They are identical to the parent tissue. Here, the cells are obtained from the source through various enzymatic or mechanical methods, and are transferred to a cell culture medium. They usually have a lesser life span.
- **Cell lines**: Cell lines are cultures of animal cells that can be propagated repeatedly and sometimes indefinitely. They arise from primary cell cultures. Primary cultures are initiated directly from the cells, tissues, or organs of animals and are typically used in experiments within a few days.

Date: 25 July 2022

Resource Person: Sir Srinivas (PhD.Scholar)

Bioinformatics

Bioinformatics is the use of computational approach to analyse, manage and store biological data. The research in biotechnology especially that involving sequence data management and drug design occurred at a speedy rate due to development of bioinformatics.

A number of tools and software are developed for analysis and interpretation of biological complexity. There are a number of applications of bioinformatics sequence analysis and alignment, molecular modelling, docking, annotation and dynamic simulation to accelerate biotechnological research. It is expected that many future bioinformatics innovations are likely to stimulate analysis of vast biological data. Here, the importance of bioinformatics in various fields of biotechnology could be high lightened such as genomics, proteomics, transcriptomics, cheminformatics, climate change studies, drug discovery and development, waste clean-up, bioenergy, crop improvement, veterinary sciences, forensic sciences and biodefense.

The NCBI (National Center for Biotechnology) houses a series of databases relevant to biotechnology and biomedicine and is an important resource for bioinformatics tools and services. Major databases include GenBank for DNA sequences and PubMed, a bibliographic database for biomedical literature. Other databases include the NCBI Epigenomics database. It also involves many tools one of the most common tool is BLAST (Basic Local Alignment search tools) .

BLAST (Basic Local Alignment search tools): Basic Local Alignment Search Tool and refers to a suite of programs used to generate alignments between a nucleotide or protein sequence, referred to as a “query” and nucleotide or protein sequences within a database, referred to as “subject” sequences.

Date: 26 July 2022

Resource Person: Dr. Deori and Dr. Himisksha Chakravarty. (ProjectAssistant)

Semen Evaluation

Biological application in livestock's production

Various biotechnology methods are used in improving the breeding stock of animals. These include artificial insemination (AI), embryo transfer (ET), in-vitro fertilisation (IVF), somatic cell nuclear transfer, and the emerging technology on somatic cell nuclear transfer.

Artificial Insemination. One of the earliest perfected technologies is artificial insemination (AI) where new breeds of animals are produced through the introduction of the male sperm from one superior male to the female reproductive tract without mating. AI reduces transmission of venereal disease, lessens the need of farms to maintain breeding males, facilitates more accurate recording of pedigrees, and minimises the cost of introducing improved genetics. Various technologies have evolved that led to the efficient use of AI in developing desired livestock, including the methods of freezing semen or cryopreservation and sperm sexing.

In-vitro Fertilisation. In case other artificial reproductive techniques fail due to difficulties such as blocked reproductive systems, non-responsive ovaries in the females, marginal semen quality and quantity in the male, and presence of disease, in vitro fertilisation (IVF) is used. The fertilisation of the sperm and the egg is conducted in vitro (outside the animal's body) at specific environmental and biochemical conditions. To date, successful IVFs have been conducted in various animal species due to advances in embryo production and cryopreservation of reproductive cells. Since the birth of the first rabbit conceived through IVF in 1959, IVF offspring have been born in mice, rats, hamsters, cats, guinea pig, squirrels, pigs, cows, monkeys, and humans.

Embryo Transfer. Embryo transfer (ET) from one mother to a surrogate mother makes it possible to produce several livestock progenies from a superior female. Selected females are induced to super ovulate hormonally and inseminated at an appropriate time relative to ovulation depending on the species and breed. Week-old embryos are flushed out of the donor's uterus, isolated, examined microscopically for number and quality, and inserted into the lining of the uterus of surrogate mothers.

Somatic Cell Nuclear Transfer. Somatic cell nuclear transfer (NF) is a technique in which the nucleus (DNA) of a somatic cell is transferred into a female egg cell or oocyte in which the nucleus has been removed to generate a new individual, genetically identical to the somatic cell donor. This technique was used to generate Dolly from a differentiated adult mammary epithelial cell which demonstrated that genes that are already inactivated in differentiated tissues can be completely reactivated.

Evaluating Boar Semem Quality

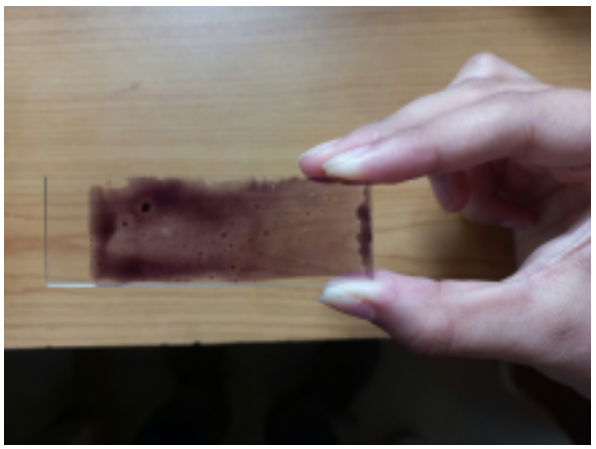
Generally, there are four basic parameters that are measured to evaluate boar semen quality: Concentration, motility, morphology and acrosome integrity. Of these,

concentration and motility are perhaps most routinely used for sorting ejaculates prior to processing since they require the least amount of time and are required to calculate semen doses/ejaculate. Measuring semen concentration or total numbers of spermatozoa is not a component of semen quality evaluation, but more so, as a tool to monitor the health and productive output of the boar and as the primary feature in processing boar ejaculates for optimizing the genetic potential of a single individual. Accurate assessment of sperm numbers is not the only factor for increasing semen doses per ejaculate and boar stud efficiency in terms of semen output.

Using Stains to Evaluate sperm morphology

Rough morphology evaluations of individual boar ejaculates are necessary, however periodic detailed exams may provide a measure of quality control to ensure that rough estimates are not grossly under or over estimating the true morphological quality of an ejaculate. To conduct a thorough morphology evaluation, stains are sometimes used to accentuate the outline of the sperm cell under a light microscope. This type of evaluation, in

contrast to a gross morphological examination, should be conducted under a higher-powered lens (1000x: oil Emerson) focused on individual sperm cells. A detailed morphology examination is performed using three separate counts; one each for headpieces, mid pieces, and tails. Glass slide smears made from a 1:1 dilution of semen in eosin-nigrosin (commercially available as a "morphology stain") stain is sufficient for differential counting of sperm head morphology. One hundred total sperm cells are counted and the percent of normal sperm cells is calculated.



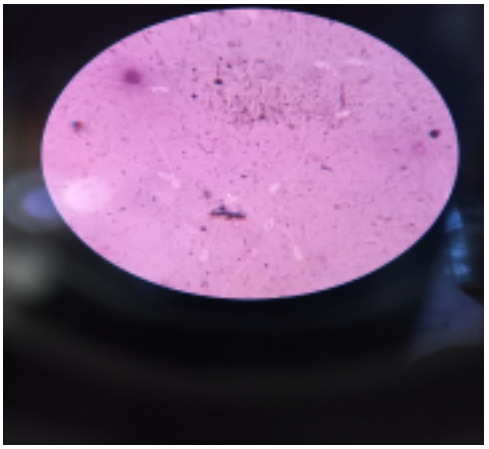


Fig: Slide preparation of sperm cells using eosin-nigrosin **Fig: Sperm cells under microscope**

Sperm abnormalities- Abnormal sperm have head or tail defects — such as a large or misshapen head or a crooked or double tail. These defects might affect the ability of the sperm to reach and penetrate an egg.

Conclusion

It was a wonderful learning experience while working on this training. This training took me through the various phases of skill development and gave me hands-on experience into the world of biotechnology under the Department of Animal and Fisheries Sciences. The various learning styles and the practical work helped us achieve the objective of this programme.

In conclusion, it is important to recognise the various fields in science that come into play in the learning process and to get exposure to the various techniques of biotechnology that has played a major role in the industrial as well as the Medical Sector.

Summer Internship Report

Sanjida Chowdhury Reya

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University ID:S2001322.

Department of Genetic Engineering and Biotechnology
Shahjalal University of Science & Technology
Sylhet-3114
Bangladesh.

জেনেটিক ইঞ্জিনিয়ারিং এন্ড বায়োটেকনোলজি বিভাগ
শাহজালাল বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়
সিলেট-৩১১৪
বাংলাদেশ।

This is certify that **Sanjida Chowdhury Reya**, a student of the Department of Biotechnology, St. Edmund's College, Shillong, India, successfully completed the internship program under the supervision of Professor Dr. Md. Abdullah Al Mamun with support by Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh from 26 July to 11 August, 2022.

I wish her future success.

11.08.2022

Dr. Md. Abdullah Al Mamun
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Executive summary:

I would like to thank Almighty for helping me to complete the internship with proper moral expedient, hard work, dedication and honesty.

During these 15 days of internship at the department of Genetic Engineering and Biotechnology in Shahjalal University of Science and Technology(SUST), I have tried to attain as much knowledge I can from the respected Professors of the department.

My main concern was to learn about basic laboratory procedures for conducting experiments and gathering ideas about several fields where biotechnology can be applied.

Acknowledgement

I, Sanjida Chowdhury Reya, would like to convey my gratitude to the Head of department of Biotechnology of St.Edmund's College, Sir Samrat Adhikari, for directing me towards this internship.

I would like to thank Professor Dr. Abdulla Al Mamun Sir for helping and guiding me at every step during this internship.

Furthermore, I would like to express my gratefulness to each and everyone who helped me to complete my internship pleasantly.

Table of contents

About the Institute:

Shahjalal University of Science and Technology (SUST), situated in Sylhet, Bangladesh was established in 1986. The University has 12 affiliated medical colleges under the School of Medical Sciences with 4000 students. Two research journals are published regularly from SUST, one in Bangla and the other one in English where a few hundred research papers are submitted every year.

Summary of the tasks done and learning:

- Standard operating procedure for the laboratory (SOPs)

SOPs incorporating safety and heath considerations must be developed and followed when laboratory work involves the use of hazardous chemicals, especially substances which includes select carcinogens, toxic chemicals.

SOPs are written instructions that details the steps to be performed during a given experimental procedures and information about potential hazards and how these hazards will be mitigated.

Consider the following information when developing SOP:

- Type, quantity and nature of the chemicals used. Note that the safety Data sheet (SDS) list information regarding potential hazards that will need to be considered, such as toxicity, flammability, reactivity, warning properties and symptoms of exposure.
- Location of use, including fume hood or other containment devices. Include specifically designated work area for particularly hazardous substances.
- Waste collection, storage and disposal requirements.

INSTRUMENTS:

❖*Multiskan SkyHigh Microplate Spectrophotometer*:

The Thermo scientific Multiskan SkyHigh Spectrophotometer is a UV/Vis microplate spectrophotometer designed to be convenient and easy to use for virtually any photometric research application, especially DNA,RNA and protein analysis as well as turbidity measurements.

❖*Real-Time PCR Systems*:

Real time PCR machine are powerful and flexible instruments, featuring 2-5 color multiplexing, advanced optical technology and precise temperature control with thermal gradients. It monitor the amplification of a targeted DNA molecule during the PCR.

Difference in Biosafety Laboratory Level 1,2,3 & 4:

- Biological Safety Level (BSL) are a series of protections related to autoclave related activities that take place in the particular biological laboratories .
- The levels, which are ranked from one to four are selected based on the agents or organisms that are being researched or worked on any given lab setting. For example, a basic lab setting specializing in the research of non lethal agents that pose lab a minimal threat to the lab worker s and environment are considered BSL-1- the lowest biosafety level. The BSL-2 covers laboratories that work with agents associate with human disease that pose a moderate health hazard

A BSL -3 laboratory typically include work on microbes that are indigenous or exotic, and can cause serious or potentially lethal disease through inhalation.

BSL-4 lab consist of work with highly dangerous or toxic microbes. Infection caused this microbes are frequently fatal; and come without treatment or vaccines.

Knowing the difference in biosafety lab level and their corresponding safety requirements is imperative for anyone working with microbes in lab setting.

COVID-19 and PCR testing:

The nose swab PCR test for COVID-19 is a reliable and accurate test for diagnosing COVID -19.

The polymerase chain reaction (PCR)test for COVID-19 is a molecular test that analyzes upper respiratory specimen, looking for genetic material(ribonucleic acid or RNA) of SARS-CoV-2, the virus that cause COVID-19. the PCR technology is used to amplify small amount of RNA from specimens into deoxyribonucleic acid (DNA) which is replicated untilSARS-2 CoV-2 is detectable if present.

There are 3c key steps to the COVID -19 test:

1.Sample Collection 2.Extraction of the genetic material from the collected sample 3.PCR: The PCR step then uses special chemicals and enzymes and a PCR machine called a thermal cycle to make million copies of a small portion of SARS-CoV-2 virus genetic material present in the test tube.

About Fisheries and Marine Biotechnology:

Fisheries is the study of freshwater, marine and/or estuarine aquatic systems that involve humans. A fisheries is any body of water with which human interact, such as in the form of fishing or swimming.

Fisheries scientist analyze the ecological health and sustainability of fisheries , how the health of fisheries affects the people who interact with them and vice versa. They assess and moniyor fish and aquatic invertebrate populations to determine ecological and economical health.

Marine biotechnology sometimes referred to as blue biotechnology , exploits the diversity found in marine environments in terms of the form, structure, physiology and chemistry of marine organisms.

Marine biotechnology is a knowledge generation and conversion process: it unlocks access to biological compounds and provide novel usages for them . By exploring and harnessing marine materials ,entirely new uses in area from the marine are likely to be found.

Already these are successful marine:-

- Origin pharmaceuticals,
- Novel industrial enzymes,
- Food ingredients ,new chemical compounds, etc.

Plant tissue culture :

Plant tissue culture is essential component of plant biotechnology. Apart from mass multiplication of elites, it also provide a mean to regenerate novel plants from genetically engineered cells.

Micro propagation-It is used to develop high quality clonal plants .

General techniques of micro propagation:

1. Explant selection
2. Sterilization of the seed
3. Explant Inoculation

4. Incubation of the explants for 10 days.
5. Adding shooting and rooting enzymes.
6. Acclimatization.

Genetic Disorder:

Genetic disorder occurs when a mutation affects our genes.

Carrying the gene doesn’t always mean we will end up getting the disease. They are many types including single-gene, multifactorial and chromosomal disorder.

What are the causes of genetic disorder:-

DNA in our gene instruct the gene to make proteins. These protein start complex cell interaction that help stay heathy.

When a mutation occurs, it affects the gene’s protein making instruction. There could be a missing protein, or the one that do function properly

Environmental factors (also called mutagens) that could lead to genetic to genetic mutation include-

- Chemical exposure.
- Radiation exposure
- Smoking
- UV exposure from the sun.

How your genes affect your health:

>around 5% of cancer are passed down through families.

>early onset of condition such as Alzheimer's disease, cancer and osteoporosis are often because there is family history of the condition.

How common are genetic disease conditions:

Approximately 6 out of 10 people will be affected by a condition which has some genetic background genetic condition can range from mild to severe.

How are genetic condition treated or managed:

Treatment and management strategies are designed to improve are designed to improve particular signs and symptoms associated with the disorders.

Health professionals can often provide supportive care, such as pain relief or mechanical breathing assistance to affected individuals.

If someone has a history of certain genetic disorder he/she should avoid the environmental factor which can accelerate the onset of the disorder.

A few disorder has been treated with gene therapy which is a very rare and expensive treatment.

Polymerase Chain reaction:

Polymerase chain reaction (PCR) is a commonly used on laboratory technique used to make many copies f particular region of DNA.

The key ingredients of a PCR reaction are TAQ polymerase(the DNA polymerase used in PCR are called TAQ polymerase), primer(a short sequence of nucleotides that provide a starting point for DNA synthesis), template DNA and nucleotides(DNA building blocks).

The ingredient are assembled in a tube along with cofactors needed by the enzyme.

The steps are:

1.Denaturation(96˚**c**): heat the reaction strongly to separate the DNA strands. This provide single stranded template for the nest steps.

2. Annealing(55-65˚C): cool the reaction so to the primers can bind to their complementary sequences on the single-stranded template DNA.

3. Extension(72˚**c**): Raise the temperature so TAQ polymerase extend the primers, synthesizing new strand of DNA.

The cycle repeats 25-30 times in a typical PCR reaction, which generally takes 2-4hours, depending on the length of the DNA region being copied.

The results of a PCR reaction are usually visualized using Gel Electrophoresis.

How bacteriophage can be used an alternate to antibiotic:

Microorganisms are developing resistance against antibiotic everyday.

With decrease in the discovery rate of antibiotic, there is a huge tension of urgent search for alternative to treat those disease.

Bacteriophages(phages) are one of the most promising alternative to antibiotics for clinical use. With antibiotic resistance crisis and urgent search for bacterial infection, phage therapy may soon fulfill it long held promise.

bacteriophages are the natural enemies of bacteria which were first used as therapeutic agents in human in 1919.

ENDOLYSIN v/s ANTIBIOTIC treatment;

Endolysin are enzymes use by bacteriophages at the end of their replication cycle to degrade the peptidoglycan of the bacterial host within resulting in cell lysis.

phages have a range of advantages compared to the antibiotic. The major advantages is their specificity for target bacteria, which significantly reduces the damages to the host normal flora, the phages are self limiting, I.e. they need specific host for continuous growth. If specific host are not available they will not last.

However, the human immune system eliminate incoming phages, posing an obstacle to their use a therapeutic agent.

Another disadvantages of phages is their narrow host range, making it difficult to look for suitably paired pages for a given bacterial pathogens.

Conclusion:

AS an undergraduate of the St Edmunds college I would like to say that it is an excellent opportunity for me to gain the ground level experience through this internship . I am grateful to SUST for giving me this opportunity.

The main objective of this training was to provide opportunity to the intern to observe and learn about the basic things related to biotechnology that is needed to apply in the real industry. I feel I got the maximum out of this internship. Also I learnt the way of work in an institute, punctuality, the importance if maximum commitment.

I am really grateful to acquire all these knowledge through this summer training program.

“Summer training

On

Basic Microbiology”

Submitted by

Abantika Goswami

Of

St. Edmunds College

Department of Biotechnology

Summer training Report

ACKNOWLEDGEMENT

I would like to express my special thanks of gratitude to my **Summer Training Programme's** guide **Sir KOBEN JOHN NONGKYNRIH**, Head of the Department **Sir Samrat Adhikari** as well as the principal **Dr. Sylvanus Lamare** who gave me the golden opportunity to do this wonderful Summer Training Program at St. Edmund's College, which provided me an opportunity explore the new horizons.

I sincerely express my gratitude towards the St.Edmund's College for providing this opportunity.

Secondly I would also like to thank my parents and friends who helped me a lot in finalizing this report within the limited time frame.

Date : 8.08.22

Abantika Goswami

Place :Shillong

Dept. of Biotechnology-III Yr.

St. Edmunds College, Shillong

TABLE OF CONTENTS

INTRODUCTION

The **Summer Training Programme** at St. Edmunds College, **Biotechnology Department** was Designed to provide the biotech interns with knowledge about the fundamentals of laboratory procedures, from a thorough run-through of all the tools in the lab to develop the research approach for a project that has been completed.

The majority of the curriculum was devoted to provide the interns with practical experience in isolating bacterial colonies from a specific source and identifying the bugs using various staining technique and biochemical testing. The same programme contained **isolation of genomic DNA** and plasmid from a particular bacteria and running **agarose gel electrophoresis** to determine the species. The application also provided accurate calculation of chemical reagent for the preparation of various medium required for the isolation procedures. The final phase of the curriculum comprised classes on **research methodology and biostatistics,** where the fundamentals and significance of biostatistics are imparted to us and the proper framework for developing a research methodology was taught.

GOOD LABORATORY PRACTICES

One of the fundamental purposes of the Principles of Good Laboratory Practice (GLP) is to ensure the quality and integrity of test data related to non-clinical safety studies. The way in which study data, supporting human, animal and environmental safety assessment, is generated, handled, reported, retained and archived has continued to evolve in line with the introduction and ongoing development of supporting technologies. However, the main purpose of the requirements of the Principles of GLP remains the same in having confidence in the quality, the integrity of the data and being able to reconstruct activities performed during the conduct of non-clinical safety studies.

There are some rules which must be observed for the successful completion of the laboratory exercise, personal safety and convenience of others working in the laboratory:

- Attending all required laboratory safety training prior to the start of the research assignment.
- Reading all procedures and associated safety information prior to the start of an experiment.
- Performing only those experiments authorized by the supervisor.
- To follow all written and verbal instructions. Ask for assistance if any guidance is required.
- Working under direct supervision at all times.
- The locations and operating procedures for all safety equipments should be known. This includes the eyewash station and safety shower.
- Knowing the locations of the nearest fire alarms and at least two ways out of the building. Never to use an elevator in emergencies.
- Being alert and proceeding with caution at all times in the laboratory. Immediately notify the supervisor of any unsafe conditions.
- Knowing the proper emergency response procedures for accidents or injuries in the laboratory.
- Practicing good personal hygiene. Washing hands after removing gloves, before leaving the laboratory, and after handling a potentially hazardous material.
- While working in the laboratory, we should wear personal protective equipment - eye protection, gloves, laboratory coat - as directed by the supervisor.
- Proper segregation and disposal of all laboratory waste.
- Dressing for work in the laboratory. Wearing clothing and shoes that cover exposed skin and protect from potential splashes. Tieing back long hair, jewelry, or anything that may catch in equipment.
- Never to eat food, drink beverages, chew gum, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
- Usage of a chemical fume hood or biosafety cabinet, as directed by the supervisor.
- Observing good housekeeping - keeping aisles clear.
- Report damaged electrical equipment to the supervisor. Do not use damaged electrical equipment.1

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DEMONSTRATION OF ALL INSTRUMENTS

Microbiology is a specialized branch of biological sciences. Microorganisms are all around us and play a special role in the ecology of life on earth. According to Louis Pasteur “Life would not long remain possible in the absence of microbes”. However, some microorganisms cause diseases in humans, other animals and plants. There is, as in all sciences, a need for basic equipment, much of which can be found in any biological laboratory. Following is a list of basic requirements which a microbiologist requires in his laboratory for microscopic examination, isolation or culturing and identification of a microorganism as well as to study its structure, function and application.

The most commonly used equipment is inoculation needles, transfer loops, inoculation, Bunsen burner, autoclave (or pressure cooker) incubators, hot air oven centrifuge, spectrophotometer magnetic stirrer electric shaker and rotary shaker heating plate, heating mantle distillation plant, UV-lamp carbon dioxide cylinder, water-bath and a single-pan balance that has weights (for general use) chemical balance, fine analytical balance pH meters, Quebec colony counter, Laminar air flow, camera lucida electrophoresis and a high-quality microscope and many more.

Here are some of the instruments that has been used in the summer training:

1. Inoculation needle and inoculation loop

- They are among the most frequently employed tools.
- The Inoculation needle/loop consists of a platinum wire that is welded to a metallic rod.
- Wire loops have an handle that is fitted with a steel screw shaft. Metallic rod nichrome, or platinum wire will be put into.
- The wire is be wrapped around a small circular object like a pencil or other similar objects. to create a loop making it twist mechanically. The loop must be designed as to keep an elongated film within it, by dips in the solution. In order to do this, a dimension (5-7 millimeters) that the wire has is suggested.
- Straight wire or straight needle is made of wire instead of loop. The free or open part of it is sharp. Straight and loop wire must be sterilized through either Bunsen burners or the hot heating coil until the loop or needle turn hot red. When the loop or wire has cooling, they are typically utilized for transferring cultures out of liquid broth.

- The straight needle is utilized to transfer the culture out of solid media. A smaller quantity of liquid culture may be controlled using a straight needle.
- The loop and wire can be are used to extract small amounts of solid material from a microbial colony and also to inoculate liquid or solid medium. Both the straight and loop wire need to be heated right after use, so that contamination is prevented.

2. Waterbath

- Waterbath is an instrument which is utilized to supply an unchanging temperature to the sample
- It's a small insulating box made of steel, and fitted with an the electrode of an electric heating coil.
- It is also controlled via the thermostat.

3. Autoclave

The killing effects of heat on living organisms can be achieved through the increase of steam pressure in an enclosed system. The water molecules are consolidated which results in an increase in their permeation. The water is boiling at 100 degrees and steam builds up in a closed vessel, leading to an increased pressure. This relationship between temperature and pressure is illustrated below.

- The autoclave is generally composed of pressure cookers composed of gun-metal sheets, which are held in the aluminum case.
- It's closed with a swing doors that are secured to the wall by bolts with a radical design.
- In laboratories for microbiology, an autoclave with a horizontal system that is jacketed is essential.
- The steam flows from below at the bottom. The walls on the sides will be heated through the jacket. It is equipped to keep track of the pressure.
- There is a way to regulate the pressure with a the pressure meter. It is a security valves that protect against any accidents. It's based on moist heat , which is can be used for sterilisation.
- The Materials required for the experiment such as the glasswares, petriplates,conical flask, haemocytometre,etc are nicely covered and autoclave to kill all the contaminants present in the equipments.

4. Laminar Air Flow

Laminar flow is an instrument that is comprised of an air compressor located in the rear of the chamber, which is able to create air flow at a the same velocity across parallel lines of flow. It has a specific filtering system that is high-efficiency particles in the air **(HEPA**) that is able to remove particles that are as small **as 0.3 millimeters.**

- In front of the blower there is a mechanism which the air that is blown out of the blower creates air velocity along flow lines that are parallel.
- The concept of laminar flow relies on the flow of an air current that is uniform in velocity through parallel flow lines, which assist in the transfer of microbial culture under an aseptic condition. Air flows through filters and then into the enclosure. the filters do not permit any type of microbes to enter the system.
- Within the chamber, one fluorescent tube and another UV tube are installed. Two switches for these tubes as well as an additional switch to regulate of airflow are installed on the device. Because of the uniform speed and the parallel flow of air current the pouring of media, plating. slant preparations, streaking etc. Without any contamination are carried out.
- At first dust particles are removed off the surfaces of flow by using a smooth cloth that is infused with alcohol. Turn on **the UV light** for a time of 30 minutes in order to eliminate germs that are found in the work space.
- Front cover of device is opened to keep the material which is to be kept within. It is set to the appropriate level so that the air in the chamber is removed as the air in the chamber could be contaminated or carry contaminants.
- All work related to pouring or plating, streaking etc. must be completed within the flame area of the burner or lamp.

5. Incubator

- An incubator is a device comprised of a steel/copper chambers, in which air or warm water circulates by electrical current or through a small gas flames.
- Incubator temperature is maintained steady due to its control via a thermostat.
- The incubator is made of a double walled chamber that can be that can be adjusted to the desired temperature. This is accomplished by an external knob to control temperature control. The space between walls then insulated to test the heat conduction. A thermometer is placed from the top to record the temperature. Nowadays, sophisticated incubators are made available with oxygen and humidity control systems.

- The temperature has a significant impact on development of microbial. So, instruments are typically constructed to permit the microorganism that is wished to thrive at a specific temperature.
- It's designed to allow the growth of microbial colonies in the appropriate medium at the right temperature. When using an incubator, difference in temperature shouldn't exceed one degree.

Hot air oven

7. Electrical balance

- It works with the help of electricity and displays a the digital weights display.
- It is comprised of a single pan that weighs a single pan. Its weight is counterbalanced with weights, and is set to zero.
- The weighted material is put on the balance pan and the counterweights required are removed using the knobs. As time passes, the digital scale starts moving between up and down.
- Always take off the counter-weights that are proportional to the material's weight.

8. Spectrophotometer (Colorimeter)

- Spectrophotometer is a tool which makes use of the light source as an instrument for radiation. It also measures changes in optical density , or absorbance.
- It is based on three fundamental principles: (i) the radiation source, (ii) a unit to disperse radiation across various wavelengths and (iii) an instrument that can will detect how much radiation is present that is detected at different wavelengths.
- Spectrophotomete employs the monochromatic (narrow frequencies) radiation, while colorimeter employs wide wavelengths.
- There are a variety of atoms as well as their electron clouds within every chromophore (a chemical molecule or a portion that is). Because of changes in the energy levels of electrons, the form of the chromophore can be altered. These variations in energy are recorded using any spectrophotometer.

9. Centrifuges

The centrifuge is a device which rotates at a high rate and separates particles or substances by the density and mass with the help by centrifugal force. The force exerted by the centrifuge is measured in terms of revolutions every minutes (rpm) of the angular speed. A centrifuge is comprised of the "head" which is rapidly rotated with an up-right motor. Usually, four

containers or cups are connected to the head to hold the tubes and other vessels made of the material that the particulate matter will be separated. When centrifugation is performed, the liquid that contains particles is kept inside tubes and moves at a specific speed, and, when the centrifugation process is completed the particles are settled on lower levels of tubes. The various types of centrifuges typically used include slow-speed (clinical centrifuge) and high-speed, also known as ultracentrifuge and superspeed. The maximum speed limits of ultracentrifuges with low-speed, high-speed or low-speed speeds are **5500 rpm, 18000 rpm** and **20000-60000 rpm** and **20000 to 60000 rpm,** respectively. They are utilized for the separation of particles in suspended matter removal of liquid mixtures with varying in density and solids or liquids the concentration of microorganisms within various samples for studies of enzymatic activity.

10. Microscope

For the study of basic and applied microbiology. The microscope is an essential tool in all the tools needed in the laboratory. The microorganisms in tiny sizes are visible only through a microscope and are inaccessible to naked eye. Some of them are colorless, but the microscope with a the phase contrast attachment can see them with high resolution. Antoni van Leeuwenhoek (1673) invented in the field of science biconcave lenses that were positioned between two plates made of steel that had a magnification of **300X** as a microscope.

The microscope was utilized by him to observe microbes. The electron microscope today is capable of magnifying over **250,000X** of the specimen. There are two main kinds of microscopes are offered that include light microscopes, which comprise dark field, bright field as well as phase contrast and electron microscopes and fluorescent microscopes, its function is based on the electron beam and magnets to view submicroscopic images. The most basic microscope is comprised of an eyes **(10X magnification)** which is inserted inside the tube of the microscope.

CHEMICAL CALCULATION AND REAGENT/MEDIA PREPARATION

For the preparation of culture media the required equipments are:

1. Peptone
2. Beef Extract
3. NaCl
4. Distilled water.
5. Agar powder

So, at first 400ml of distilled water is taken into a conical flask of 1000ml. To which measured amount of peptone, beef extract, NaCl and Agar are to be taken.

Nutrient Broth and Agar*

The amount of **peptone** taken in 1000ml of distilled water is 5g

Therefore, in 1ml water the amount of **peptone** taken is = 5/1000g
So, the amount of **peptone** taken in 400ml of water = 5/1000g×4000 = 2g

The amount of **Beef exatract** taken in 1000ml of distilled water is 3g

Therefore, in 1ml water the amount of **Beef extract** taken is = 3/1000g
So, the amount of **Beef extract** taken in 400ml of water = 3/1000g×4000 = 1.2g

The amount of **NaCl** taken in 1000ml of distilled water is 5g

Therefore, in 1ml water the amount of **NaCl** taken is = 5/1000g
So, the amount of **NaCl** taken in 400ml of water = 5/1000g×4000 = 2g

The amount of **Agar** taken in 1000ml of distilled water is 15g

Therefore, in 1ml water the amount of **Agar** taken is = 15/1000g
So, the amount of **Agar** taken in 400ml of water = 15/1000g×4000 = 6g

*For nutrient agar we have added 6g agar. **Mainly used for isolation of bacteria and actinomycetes.**

After measuring the amount of the ingridients, these are poured into the conical flask filled with 400ml of water. The solution is mixed and then heated into the microwave for several minutes at high temperature until dissolved completely. A broth is formed.

Culture media is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible, and repeatable microbiological test results is reduced. A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms. Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents.

ISOLATION OF MICROORGANISM

Requirements:

- Sample (soyong squash) in a sterile bottle
- Sterile Petri-plates
- Micropipette
- Nutrient Agar media
- Test tubes

The Petri-plates, the culture media and Micropipette is taken into the Laminar Flow (We should first Make sure that the Laminar Flow is Cleaned properly with **70% Ethanol** And **UV light** is switched on and kept for 15 mins to kill any kinds of microbes present in it).

The nutrient culture media is poured into the Petri-plates covering 2/3rd of it. We have to make sure that the plates are covered with lid instantly after pouring the media so that it is not attacked by microbes.

After pouring, the plates are to be kept inside the laminar flow until it solidifies. In the mean time, the serial dilution method is applied. After the dilution, small amount of the sample as well as diluted media is taken in each plates and then inoculated. Bacterial culture growth is observed.

Culture media has been used by microbiologists since the nineteenth century. Even with the increased use of rapid methods the majority of techniques found in the pharmaceutical quality control laboratory require growth media. For the assessment of culture media, no one definitive standard exists.
Culture media is of fundamental importance for most microbiological tests: to obtain pure cultures, to grow and count microbial cells, and to cultivate and select microorganisms. Without high-quality media, the possibility of achieving accurate, reproducible, and repeatable microbiological test results is reduced. A microbiological culture medium is a substance that encourages the growth, support, and survival of microorganisms. Culture media contains nutrients, growth promoting factors, energy sources, buffer salts, minerals, metals, and gelling agents.
Since there are many types of microorganisms, each having unique properties and requiring specific nutrients for growth, there are many types based on what nutrients they contain and what function they play in the growth of microorganisms.

SERIAL DILUTION

Serial dilution is a laboratory technique, in which a stepwise dilution process is performed on a solution with an associated dilution factor. In the laboratory, this method is used to decrease the counts of viable cells within a culture to simplify the operation.

In serial dilution, the cell count or density gradually decreases as the serial number increases in each step. This makes it easier to calculate the cell numbers in the primary solution by calculating the total dilution over the whole series.

The following is the serial dilution procedure for a ten-fold dilution of a sample to a dilution factor of 10-6:

1. 10 sterile and clean test tubes are taken.
2. The selected sample(soyong squash) is taken into a test tube and the remaining test tubes are filled with 9 ml of distilled water.
3. 1ml of sample into the sterile pipette is drawn. The sample must be properly mixed, if necessary use a vortex meter.
4. Then this 1ml sample is transferred within the first test tube to make the total volume of 10 ml. It provides an initial dilution of 10-1. It should be made sure during the transfer, the tip of pipette doesn't touch the wall of test tube or no amount of sample remains at the tube wall.

In serial dilution technique, the dilution factor can be calculated either for a single test tube or for the entire series (total dilution factor).

In the case of ten-fold dilution, where 1ml of sample is transferred to 9 ml of diluent, the dilution factor for that test tube will be:

$$\text{Dilution factor} = 1\text{ml}/1\text{ml} + 9\text{ml} = 1/10$$

The samples and the diluted samples are marked as 10^{-1},10^{-2}……..10^{-9}. Then with the help of a pipette 1ml of the sample and the diluted sample is taken and spread in the solidified nutrient media. After this the plates are placed inside the incubator for incubation.

PURIFICATION OF MICROORGANISMS

Streak-plate method

The streak-plate method offers a most practical method of obtaining discrete colonies and pure cultures. It was originally developed by two bacteriologists, **Leoeffler** and **Graffkey** in the laboratory of Robert Koch. In this method, a sterilized loop is dipped into a suitable diluted suspension of organisms which is then streaked on the surface of an already solidified agar plate to make a series of parallel, non-overlapping streaks.

Requirements

1. Isolated sample
2. Nutrient agar plates
3. Inoculating loop
4. Bunsen burner.

Procedure

1. The inoculating loop was sterilized in the Bunsen burner by putting the loop into the flame until is it red hot.
2. An isolated colony is picked from the agar plate culture and was spread over the first quadrant.
3. Immediately the inoculating loop is streaked very gently over a quarter of the plate using a back and froth motion.
4. The loop is flamed again and allowed to cool. Going back to the edge of area 1 that was just streaked, the streaks were extended into the second quarter of the plate.
5. The loop is flamed and allowed to cool. Going back to the area that was just streaked, the streaks were extended into the third quarter of the plate.
6. The loop is flamed again and allowed it to cool. Going back to the area that was just streaked, the streaks were extended into the center fourth of the plate.
7. The loop is flamed once more.

STAINING TECHNIQUES

Gram staining of bacteria

The Gram stain, a differential method was developed by Dr. Hans Christian Gram, a Danish physician, in1884 that is why Gram staining. In this process, the fixed bacterial smear is subjected to four different reagents in the order listed: crystal violet, iodine solution, alcohol and safranin. The bacteria which retain the primary stain (appear dark blue or violet) (i.e. not decolorized when stained with Gram's method) are called gram-positive, whereas those that lose the crystal violet and counter stained by safranin (appear red) are referred to as gram-negative.

Requirements

1. Gram staining reagents:

   Crystal violet
   Gram's iodine solution
   95 percent ethyl alcohol
   Safranin
2. Staining tray
3. Wash bottle of distilled water
4. Droppers
5. Inoculating loop
6. Glass slides
7. Blotting paper
8. Lens paper
9. Bunsen burner
10. Microscope

Procedure

1. Thin smears of isolated sample was made on separate glass slides.
2. The smears was air dried.
3. The smears was held using slide rack.
4. The slides was covered with crystal violet for 30 seconds.
5. Each slide was washed with distilled water for a few seconds, using wash bottle.
6. Each smear was covered with Gram's iodine solution for 60 seconds.

7. The iodine solution is washed off with 95 percent ethyl alcohol. Ethyl alcohol was added drop by drop, until no more colour flows from the smear.
8. The slides was washed with distilled water and drained.
9. Saffranin is added to smears for 30 seconds.
10. It was then washed with distilled water and blot dry with absorbent paper.
11. The stained slides were allowed to air dry.

Gram Negative Bacterial Preparation

1. Harvesting of cells
   1.5ml of an overnight bacterial broth culture were pelleted by centrifuging for 2 minutes at 12,000-16,000 g. The culture medium were removed and discarded.

2.Resuspend cells

The pellet were resuspended thoroughly in 180 microlitre of Lysis Solution.

3. Prepare for cell lysis
   20 microlitre of the Protinase K solution were added to the sample. It was then mixed and incubated for 30 minutes at 55degree celcius.
4. Lysis cell
5. 20 microlitre of lysis Solution, were added, vortex thoroughly and incubated at 55 degree celcius for 10 minutes.

Bacterial spore staining

Endospore staining is the type of staining to recognize the presence spore in bacterial vegetative cells. Within bacteria, endospores are protective structures used to survive extreme conditions, including high temperatures making them resistant to chemicals.

Requirements

1. Isolated sample
2. Malachite green
3. Safranin
4. Staining tray
5. Glass slides
6. Inoculating loop
7. Blotting paper

8. Spirit lamp
9. Microscope.

Procedure

1. Smears of isolated sample was made on separate clean slides.
2. The smears was air dried.
3. The smears was flooded with malachite green.
4. The slides were steamed for 5 minutes, adding more stain to the smear from time to time.
5. The slides was washed under running tap water.
6. It was then counterstained with safranin for 30 minutes.
7. The smear was washed with diluted water
8. Blot dry slides with blotting paper.

BIOCHEMICAL TEST

Catalase Test

This test demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide.

Procedure

1. A loop was used to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. A drop of 3% hydrogen was placed in a glass slide.
3. The evolution of oxygen bubbles was observed.

Oxidase Test

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme.

Procedure

1. Strip of Whatman's No. 1 filter paper was soaked in 1% solution of tetramethyl-p-pheneylene-diaminedihydrochloride and let it dry.
2. A sterile loop was used to pick a well-isolated colong from a fresh bacterial plate and rub it onto treated filter paper.
3. Color changes was observed.

ISOLATION OF GENOMIC DNA

Principle

Isolation of genomic DNA is one of the most important and common experiment that is carried out in molecular biology and includes the transition from cell biology to molecular biology. The most common method of isolating genomic DNA without the use of commercial kit is by phenol/chloroform method. So, the basic objective of this test is to study the method of isolation genomic DNA from E.Coli.

***Reagents and chemicals**:*

- Tris base
- Proteinase K
- Phenol/chloroform(1:1)
- 200 proof ethanol
- RNAse
- Ethanol
- SDS
- EDTA
- Tryptone

- Yeast Extract
- NaCl
- LB Medium (1%tryptone, 0.5% yeast extract, 200 mM NaCl)
- TE buffer (10mM Tris-Cl (pH 8.0), I mM EDTA (pH 8.0))

Equipment

- Lysis buffer(10ml)(9.34ml TE buffer, 600 ul of 10% SDS, 60µl of Proteinase K(20 mg/ml))
- Tabletop centrifuge (Eppendorf)
- 1.5ml Eppendorf tube
- Incubator & Gloves

Procedure

- At first 1.5ml of overnight E.coli culture that was grown in LB medium is taken and transferred into a 1.5ml of Eppendorf tube and then centrifuged at maximum possible speed for about 1 min to extract the cell pellet.
- The supernatant is then discarded and the pellet is resuspended in 600 microliter of lysis buffer and completely vortexed to mix it properly.
- It is incubated for 1 hour at 37ºC. Equal volume of phenol/chloroform is added and mixed properly.
- It is then spun at maximum speed for about 5 min which leads to the formation of a white layer in the aqueous phenol/chloroform interface.
- The aqueous phase is then transferred to a new tube very carefully through 1ml pipette. The above two steps can be repeated till the white layer disappears. To remove the phenol equal volume of chloroform is added to the aqueous layer.
- The mixture is mixed properly and spun at maximum speed for about 5min. Then the aqueous layer is transferred to a new tube.
- For precipitation of the DNA, 2.5ml of cold ethanol is added and mixed properly. Precipitation may cause diffusion. The tube requires to be kept at -20ºC for about 30min and then spun. This way one will be able to see the DNA pellet.
- It then spun for about 15min at 4ºC. The supernatant is discarded and the DNA pellet is rinsed with 1ml of 70% ethanol.
- Then again it is spun at maximum speed for about 2 min and the supernatant is discarded while the DNA pellet is washed with 1ml of 70% ethanol.
- Now it's again spun at maximum speed.
- At last the DNA is resuspended in TE buffer.

ISOLATION OF PLASMID DNA

Principle

In prokaryotes, plasmid is double stranded, circular, and is found in the cytoplasm. The cell membranes must be disrupted in order to release the plasmid in the extraction buffer. Solution 1 contains glucose, Tris, and EDTA. Glucose provides osmotic shock leading to the disruption of cell membrane, Tris is a buffering agent used to maintain a constant pH8. Plasmid can be protected from endogenous nucleases by chelating Mg2++ ions using EDTA. Mg2++ ion is considered as a necessary cofactor for most nucleases. Solution II contains NaOH and SDS and this alkaline solution is used to disrupt the cell membrane and NaOH also denatures the DNA into single strands. Solution III contains acetic acid to neutralise the pH and potassium acetate to precipitate the chromosomal DNA, proteins, along with the cellular debris. Phenol /chloroform is used to denature and separate proteins from plasmid. Chloroform is also a protein denaturant, which stabilizes the rather unstable boundary between an aqueous phase and pure phenol layer. The denatured proteins form a layer at the interface between the aqueous and the organic phases which are removed by centrifugation. Once the plasmid DNA is released, it must be precipitated in alcohol. The plasmid DNA in the aqueous phase is precipitated with cold (0o C) ethanol or isopropanol. The precipitate is usually redissolved in buffer and treated with phenol or organic solvent to remove the last traces of protein, followed by reprecipitation with cold ethanol.

Materials Required

Luria Broth

Bacterial cells containing plasmid

Reagents TE buffer (pH 8.0)

Solution I

Solution II

Solution III

Phenol-chloroform mixture

Isopropanol

70% ethanol

1% agarose gel and an electrophoresis apparatus

Autoclaved Distilled Water

Eppendorf tubes 2 ml

Micropipette

Microtips

Microfuge

Preparation of Reagents:

1. TE BUFFER (pH 8.0): 10 mm Tris HCl (pH 8.0) 1 mm EDTA (pH 8.0)

2. Solution I: Lysis solution: 50 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 mg/mL RNAse A, (store at 4˚C)

3. Solution II: Denaturing solution: 0.2 M NaOH; 1 % (w/v) SDS

4**. Solution III: Neutralizing solution**: 3 M Potassium acetate, pH 5.5

5. **PHENOL – CHLOROFORM MIXTURE**: Mix equal volume of phenol with chloroform. Keep the mixture on ice and add 20 ml TE buffer, extract by shaking for 15 minutes. Remove the dust on the surface layer using a pipette. Repeat 4-5 times. Add 30-40 ml of TE buffer and store it in dark.

6. Isopropanol

PROCEDURE:

- 2ml of overnight culture is taken and centrifuged for 5 minutes. The supernatant is then discarded carefully.
- 100µl of solution I is added to the cell pellet and resuspended by mixing gently.
- The above mixture is incubated at room temperature for 5 minutes.
- Thereafter 200µl of solution II is added to the mixture and mixed by inverting the tube.
- The above mixture is then incubated at room temperature for 5-10 minutes.
- Then 500µl of ice cold solution III is added to the mixture and mixed by inverting the tube.
- The mixture is incubated on ice for 10 minutes.
- The above step is followed by centrifugation of the mixture at 10.000 rpm for 5 minutes.

- The supernatant is transferred into a fresh tube.
- 400µl of phenol-chloroform mixture is added to the contents, and mixed well by inverting and incubating them at room temperature for 5 minutes.
- Again the mixture is centrifuged at 10.000 rpm for 5 minutes.
- The supernatant (viscous) is collected using cut tips and transferred to a fresh tube.
- 0.8 ml of isopropanol is added and mixed gently by inversion.
- The mixture is incubated at room temperature for 30 minutes.
- Centrifuge the contents at 10,000 rpm for 10 minutes.
- The supernatant is discarded after the centrifugation.
- This is followed by air drying for 5 minutes, and addition of 100µl of TE buffer or autoclaved distilled water to the pellet to resuspend the plasmid DNA.
- The DNA is run on the agarose gel.

AGAROSE GEL ELECTROPHORESIS

Introduction

Agarose gel electrophoresis is a powerful and widely used method that separates molecules on the basis of electrical charge, size, and shape. The method is particularly useful in separating charged biologically important molecules such as DNA (deoxyribonucleic acids), RNA (ribonucleic acids), and proteins. Agarose forms a gel like consistency when boiled and cooled in a suitable buffer.

Principle

The agarose gel contains molecule sized pores, acting like molecular sieves. The pores in the gel control the speed that molecules can move. DNA molecules possess a negative charge in their backbone structure due to the presence of PO4 - groups thus this principle is exploited for its separation. Smaller molecules move through the pores more easily than larger ones. Conditions of charge, size, and shape interact with one another depending on the structure and composition

of the molecules, buffer conditions, gel thickness, and voltage. Agarose gels are made with between 0.7% (provides good resolution of large 5–10 kb DNA fragments) and 2% (good resolution for small 0.2–1 kb fragments). The gel setup provides wells for loading DNA in to it. The loaded DNA molecules move towards the positively charged electrode (anode) and get separated along the length of the gel. Ethidium bromide (EtBr), a chromogen is added to the gel to visualize the separated DNA under UV transillumination. EtBr intercalates between the bases and glows when UV radiation is passed through the gel.

Purpose of gel loading buffer

The loading buffer gives colour and density to the sample to make it easy to load into the wells. Also, the dyes are negatively charged in neutral buffers and thus move in the same direction as the DNA during electrophoresis. This allows you to monitor the progress of the gel. The gel loading dye possesses bromophenol blue and xylene cyanol. Density is provided by glycerol or sucrose. Xylene xyanol gives a greenish blue colour while bromophenol blue provides bluish colored zone. The successful DNA run is determined by the presence of both the colored dye in the gel.

Materials Required

- Electrophoresis buffer: 1x TAE buffer
- Agarose ultra-pure (DNA graded)
- Electrophoresis tank, gel tray, sample comb and power supply
- Plastic or insulation tape
- Ethidium bromide: 10 mg /ml stock solution
- 5x Gel loading dye
- DNA marker solution, DNA sample and gloves.

PROCEDURE

Making a 1% Agarose Gel

- 0.5g of agarose is weighed and dissolved in 50ml of 1× TAE Buffer. (Note: A 250 ml of conical flask is used for the preparation to prevent wastage by spillage during the heating process.)
- The solution is heated over a hot plate to a boiling constituency marked with a clear solution.

• The solution is left to cool and 2µl of EtBr solution is added and mixed well by gently swirling.

• The solution is poured in the gel tray-comb set up. It is also made sure that the gel plates have been secured with tapes and the well combs are present prior to pouring.

• The solution is allowed to cool and harden to form gel.

2. Loading of Samples

• The gel is carefully transferred to the electrophoresis tank filled with 1× TAE buffer.

• The samples are prepared [8µl of DNA sample (0.1ug to 1ug) and 2ul of 5× gel loading dye].

• The comb is removed and the sample is loaded.

• Appropriate electrodes are connected to the power pack and run at 50-100 volts for 20 minutes.

• The progress of the gel is monitored with reference to tracking dye (Bromophenol blue). The run is stopped when the marker has run 3/4th of the gel.

3. Examining the gel

• The gel is placed on the UV-trans illuminator and checked for orange coloured bands in the gel.

INTRODUCTION TO BIOINFORMATICS

Definition

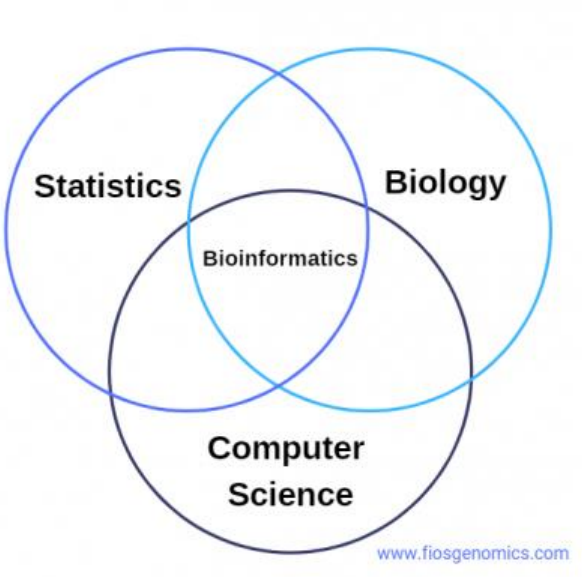
Bioinformatics is defined as the application of tools of computation and analysis to the capture and interpretation of biological data. It is an interdisciplinary field, which harnesses computer science, mathematics, physics, and biology.

Bioinformatics is essentially big data analysis for **biological** data sets. It requires **computational** and **statistical** analyses in order to extract meaning from biological data. Since this is the case, bioinformatics can also be used to refer to the development of the software

and methods which are used to understand biological data. As you will now understand, bioinformatics is an interdisciplinary field where **biology, computer science,** and **statistics** meet.

Brief Summarization

- Bioinformatics is the application of tools of computation and analysis to the capture and interpretation of biological data
- Bioinformatics is essential for management of data in modern biology and medicine
- The bioinformatics toolbox includes computer software programs such as BLAST and Ensembl, which depend on the availability of the internet
- Analysis of genome sequence data, particularly the analysis of the human genome project, is one of the main achievements of bioinformatics to date
- Prospects in the field of bioinformatics include its future contribution to functional understanding of the human genome, leading to enhanced discovery of drug targets and individualised therapy.



Brief History

Paulien Hogeweg and Ben Hesper first coined the term 'bioinformatics' in the early 1970s and defined it as 'the study of informatic processes in biotic systems'[1]. However, before the term was coined the stage was set for bioinformatics to emerge as a new field of study in the 1960s when computational methods were applied to protein sequence analysis by Margaret Dayhoff. In fact, Dayhoff has been referred to as 'the mother and father of bioinformatics.

The Components of Bioinformatics

- Biology
  The statistical analyses you conduct will have no biological basis if you do not have an understanding of biology. While it might be useful to know that the Treatment A group has results showing higher values of Protein X than the Treatment B group, you need biological knowledge to understand the pathways or genes that cause that result. Is it due to an up-regulation of a certain pathway?
  You may find that without the biological knowledge to interpret your results, you know an ending without explanation and also without the method and mechanism of action.

- Computer Science
  Without computer science, analysis speed would be significantly slower. Bioinformaticians write software to help automate analyses (instead of manually working through each one). Being able to combine hundreds of thousands of data points into analysis through coding allows for larger datasets to be created while still keeping the analysis time to a reasonable length. Depending on the size of the dataset, however, there can still be large computational time requirements, although it will be much reduced compared to a non-bioinformatic approach.
- Statistics
  In short, without statistics, there would be no in-depth analysis. Basic graphs may show that Treatment A has results with higher values of Protein X than Treatment B, but the actual significance of these results would be unknown. For example, is there a significant difference between A and B, or does it just look that way from the graph? New treatments need solid evidence behind them for approval and statistical analyses can prove the true benefits of a treatment.

Need for Bioinformatics

The field of bioinformatics has advanced considerably in the 50 years since the term was first coined. It has evolved to keep up with progress in molecular biology and computer science. The rise of the internet in the 1990s coupled with the creation of next-generation sequencing (NGS)

technologies in the 2000s created a boom in the availability of biological data to be analyzed. In turn, this led to the swift production of new bioinformatics tools.

Nowadays, we can apply bioinformatic analyses to various biological data sets such as:

- Genome Sequence Data
- Gene Variation Data
- Gene Expression Data
- Single-Cell Data
- Proteomics Data
- Metabolomics Data
- Epigenetics Data

RESULTS

1 .BACTERIAL GROWTH:

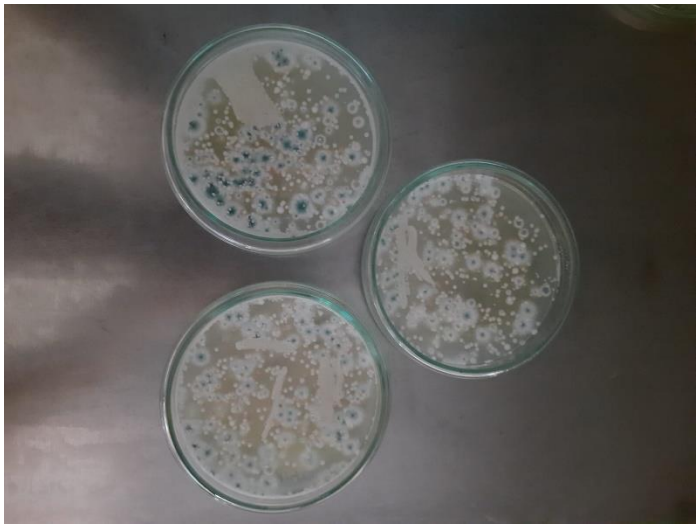


Fig: Bacterial growth is seen after incubated for 2 days.

2. PURIFIATION OF MICROORGANISMS :

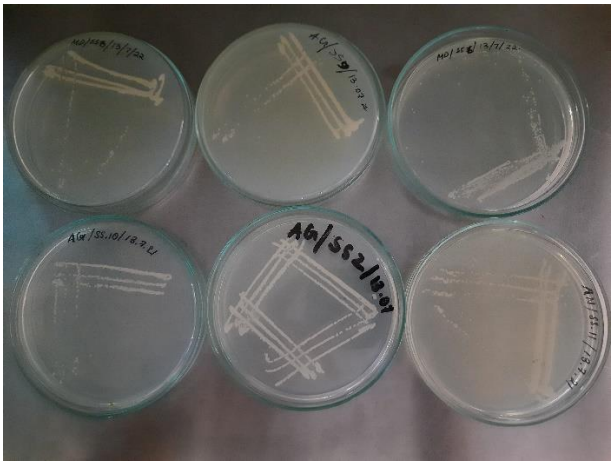


Fig: streak plate method

In the first area of streaking, there is heavy growth with fused colonies , and gradually there are fewer colonies in subsequent streaks giving a few well-isolated colonies in the final streak.

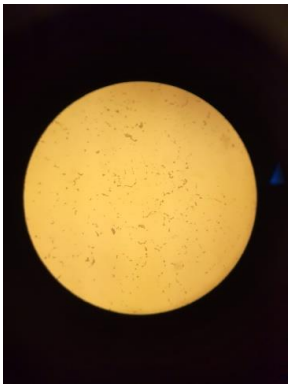
3. STAINING TECHNIQUES:

Gram staining of bacteria

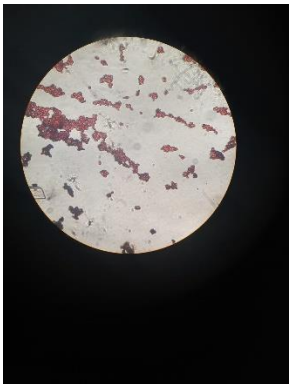
Table:

Culture	Color	Shape	Inference
SS1	Red	Coccoid	Gram negative
SS2	Red	Coccoid	Gram negative
SS3	Red	Coccoid	Gram negative
SS4	Red	Coccoid	Gram negative
SS5	Red	Coccoid	Gram negative
SS6	Red	Coccoid	Gram negative

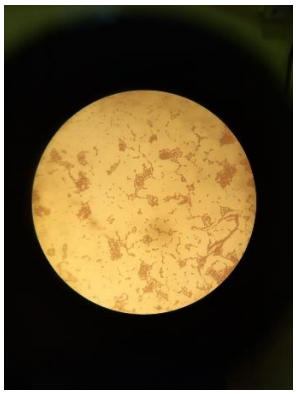
The slides which were observed under the microscope are as follows:



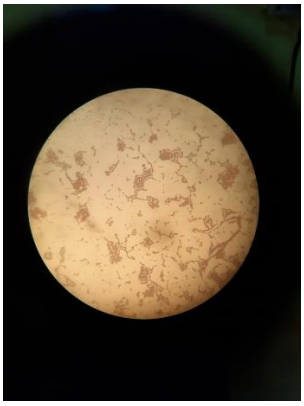
Slant culture 1



Slant culture 2



Slant culture 3



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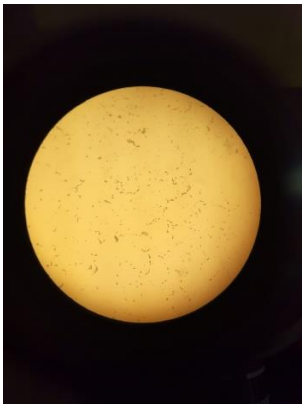
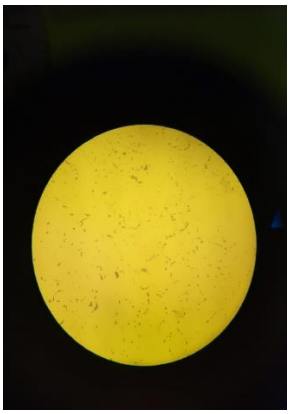
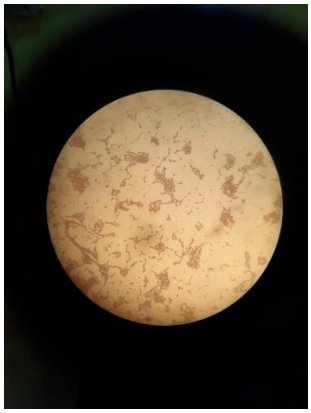


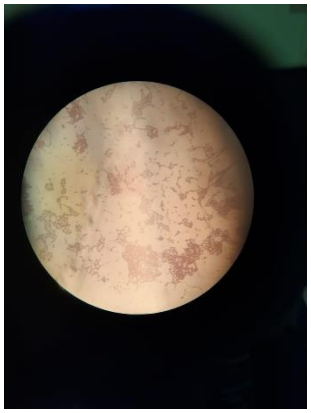
Slant culture 5



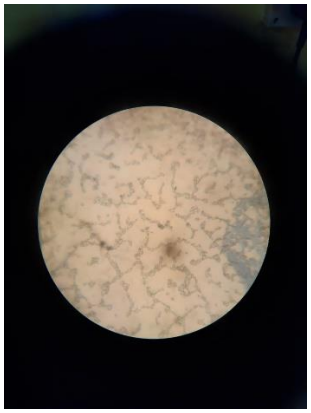
Slant culture 6



Slant culture 7



Slant culture 8



Slant culture 10

All the slides observed under the microscope were Gram negative and coccoid in shape.

Bacterial spore training

The slides which were observed under the microscope are as follows:

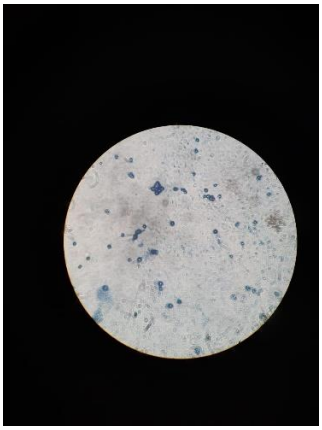


Fig: Slant culture 1

Fig: Slant culture 2

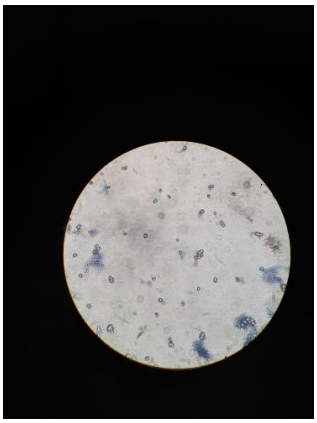
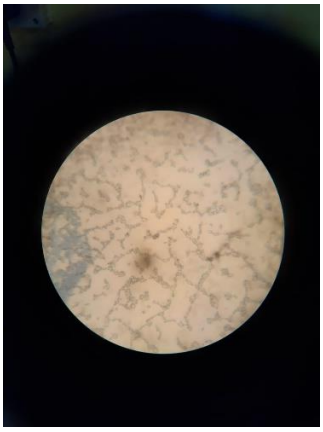


Fig: Slant culture 3



Slant culture 4

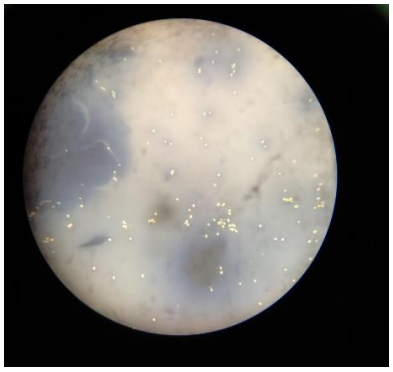


Fig: Slant culture 5

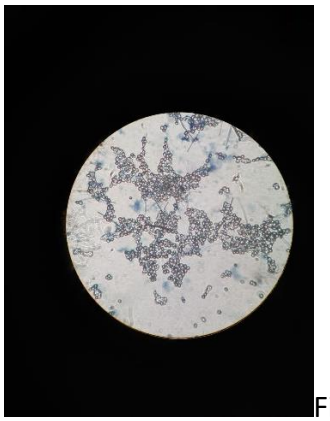
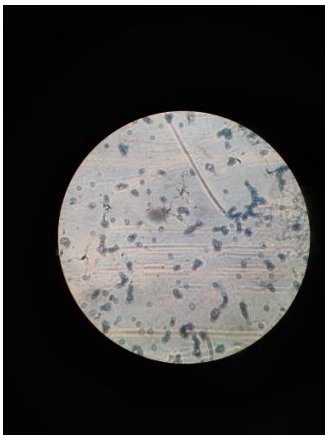


Fig: Slant culture 6



Slant culture 7

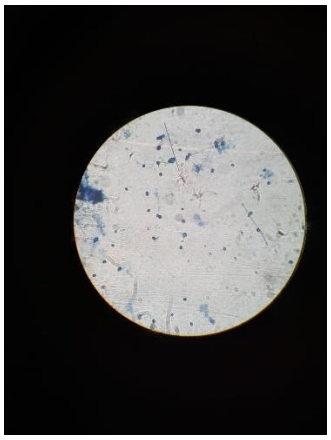


Fig: Slant culture 8

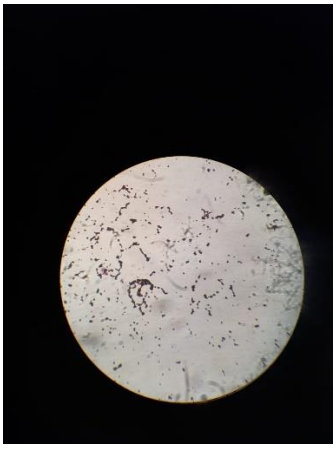


Fig: Slant culture 9

All the samples observed under the microscope showed positive results for spores.

4. BIOCHEMICAL TESTS:

Catalase Test

Slant culture (SC)	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9
Oxidase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

Evolution of bubbles was observed in all the cultures.

Oxidase Test

Slant culture (SC)	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9
Oxidase	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

A positive reaction is indicated by an intense deep-purple hue ,appearing within 5-10 seconds.

5. AGAROSE GEL ELECTROPHORESIS:

The isolated genomic DNA as well as the Isolated Plasmid DNA were observed under a UV-trans illuminator after performing gel electrophoresis on both respectively.

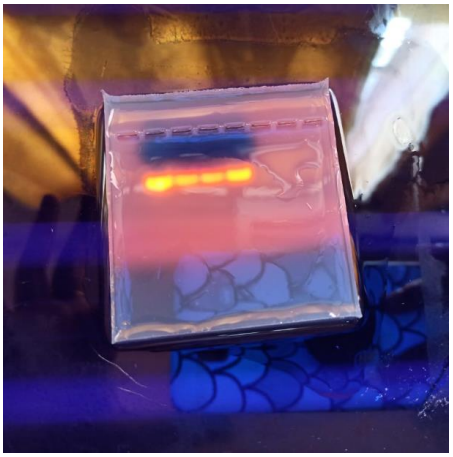


Fig. DNA bands observed under UV-trans illuminator after agarose gel electrophoresis.

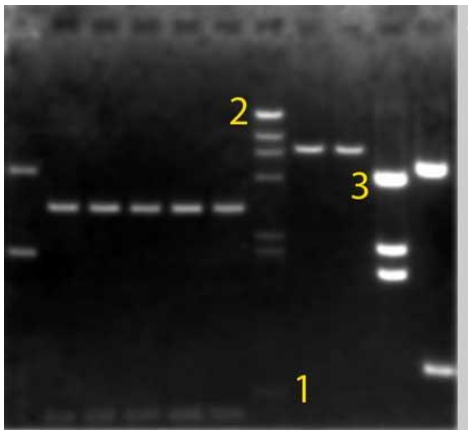


Fig. Plasmid DNA observed under UV-trans illuminator after performing agarose gel electrophoresis.

DISCUSSION

Microbiology is a branch of biological science that studies microorganisms (also known as microbes), which are microscopic unicellular or cell-cluster organisms and infectious agents.

The different types of microbes studied by microbiologists include bacteria, archaea, viruses, eukaryotes, fungi, prions, protozoa and algae. These microbes can differ dramatically in terms of size and characteristics.

Though microbes often carry negative connotations due to the association of certain microbes with diseases, many other microbes carry several benefits. For example, microbes underpin processes such as industrial fermentation (which is used to make useful products such as alcohol, vinegar and dairy products) and antibiotic production. They also act as molecular vehicles to transfer DNA to complex organisms such as plants and animals.

Microbes are vitally important to all life on Earth. As versatile organisms, they play a major role in various biochemical processes such as biodegradation, biodeterioration, climate change, food spoilage, epidemiology and biotechnology.

By applying microbes in a range of controlled settings, microbiologists can harness their power for beneficial use in areas as diverse as healthcare, food production and agriculture.

In medicine alone, microbiologists have contributed to some of history's most important scientific breakthroughs. Edward Jenner invented the world’s first smallpox vaccine. Robert Koch identified the causes of cholera, tuberculosis and anthrax. Alexander Fleming discovered penicillin. And, more recently, Barry Marshall identified the link between Helicobacter pylori infection and stomach ulcers. Microbiologists are pushing the envelope of science and helping to save lives in the process.

Introduction

Chromatographic technique

Chromatographic technique is based on separation of compound with the help of mobile phase as well as stationary phase either by adsorption or partition.

The **major types of chromatographic techniques** are Paper Chromatography, High Liquid Performance Chromatography, Gas Chromatography, Ion Exchange Chromatography and Electrophoresis.

Types of Chromatography

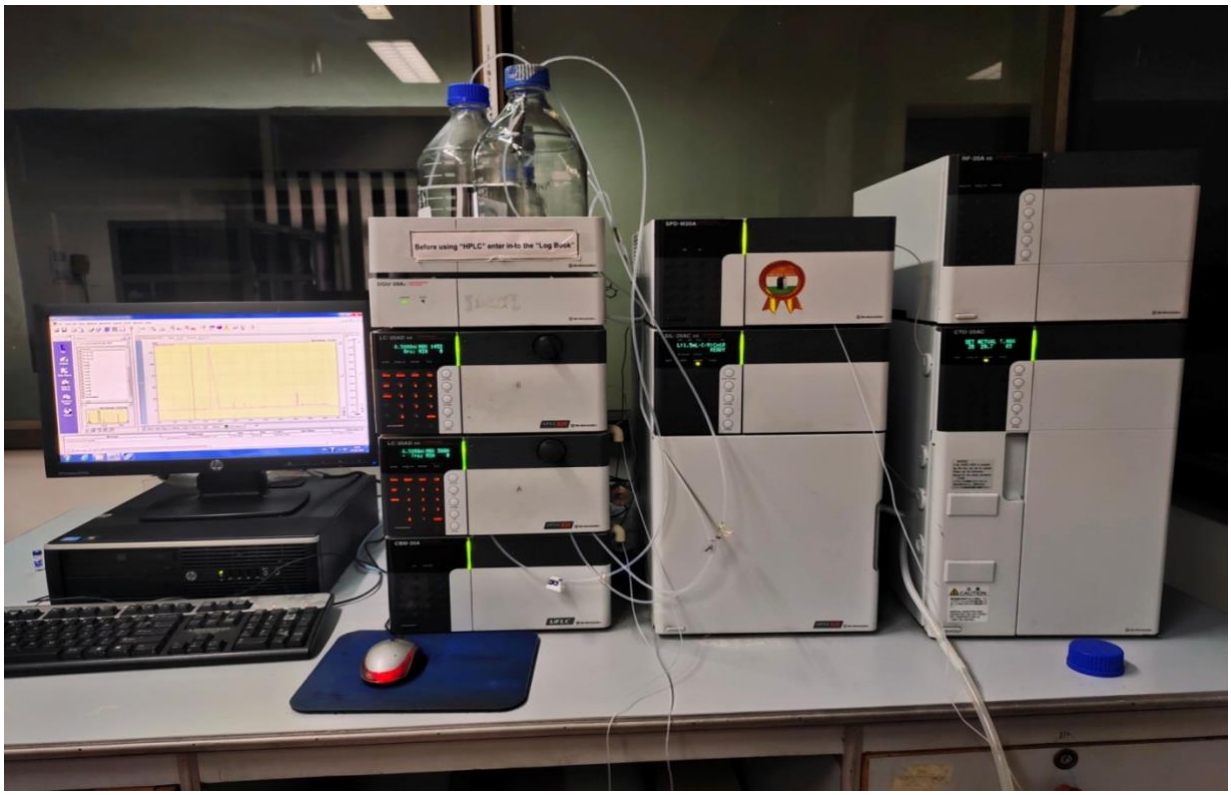
Adsorption Chromatography: Adsorption Chromatography involves the separation of a chemical mixture based on the interaction of the adsorbate with the adsorbent. In this process, the mixture of gas or liquid gets separated on the adsorbent bed that adsorbs different compounds at different rates.

Thin Layer Chromatography: Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel

Column Chromatography: It is a precursory technique used in the purification of compounds based on their hydrophobicity or polarity. In this chromatography process, the molecule mixture is separated depending on its differentials partitioning between a stationary phase and a mobile phase.

Partition chromatography: Partition Chromatography technique is defined as. the separation of components between two liquid phases viz original solvent and the film of solvent used in the column

HPLC: HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



Principle of HPLC

The sample component which has less affinity towards stationary phase will elute faster and the sample component which has more affinity towards stationary phase will elute later.

Parts of a HPLC include:

- Degasser
- Pump
- Sample injector
- Column (Stationary Phase)
- Detector: UV- visible detector (PDA)

The Pump

The development of HPLC led to the development of the pump system. The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from

the solvent reservoir into the system. High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate. Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Injector

An injector is placed next to the pump. The simplest method is to use a syringe, and the sample is introduced to the flow of eluent. The most widely used injection method is based on sampling loops. The use of the autosampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

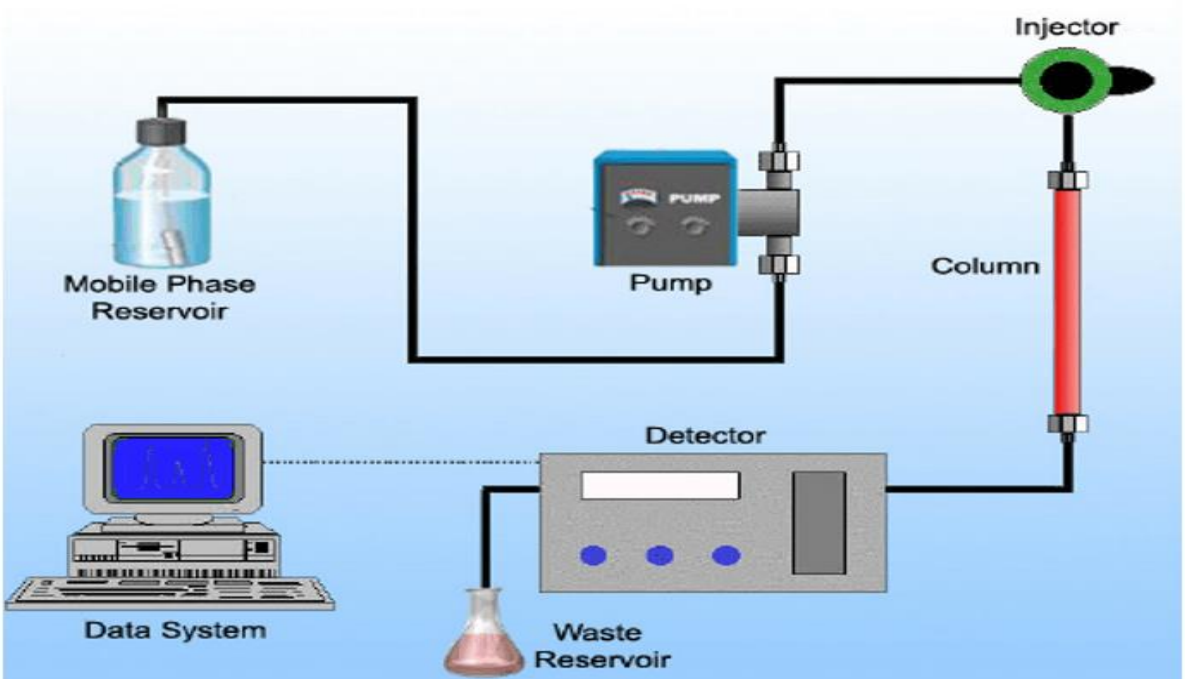
The separation is performed inside the column. The recent columns are often prepared in a stainless-steel housing, instead of glass columns. The packing material generally used is silica or polymer gels compared to calcium carbonate. The eluent used for LC varies from acidic to basic solvents. Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector

Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation. The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences. This difference is monitored as a form of an electronic signal. There are different types of detectors available.

Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes. When gas is present in the eluent, this is detected as noise and causes an unstable baseline. Degasser uses special polymer membrane tubing to remove gases. The numerous very small pores on the surface of the polymer tube allow the air to go through while preventing any liquid to go through the pore.



Types of columns used in HPLC

There are different types of columns used in HPLC

1. Normal phase columns
2. Reverse phase columns
3. Ion exchange columns
4. Size exclusion columns

Types of column

Parameters needed for method development in HPLC:

- Mobile phase selection
- Stationary phase selection
- Temp of column oven
- Flow rate
- pH of mobile phase
- Run time

Two Major Chromatographic Techniques used in HPLC:

It is based on nature of mobile phase and stationary phase.

Reverse phase chromatography: In Reverse phase chromatography, Mobile phase is polar and stationary phase is non polar, Polar compounds will elute faster. This is more commonly used technique as there is abundance of polar compounds in nature.

Normal phase chromatography: In normal phase chromatography, Mobile phase is non-polar and stationary phase is polar, non-polar compounds will elute faster.

Retention time

The time taken for a particular compound to travel through the column to the detector is known as its retention time. Different compounds have different retention times.

Steps Involved in HPLC Analysis:

- Sample collection: solid, semisolid or liquid
- Storage and handling of the samples
- Sample preparation and storage
- Sample application in HPLC/ Aim of analysis
- Data interpretation
- Preparation of report
- Submission of report

Storage and handling of the samples:

Frozen storage	-20 °C to -80 °C
Freezing temperature	-4 °C to 2 °C
Cold temperature	2 °C to 8 °C
Cool temperature	8 °C to 15 °C
Room temperature	20 °C to 25 °C
Warm temperature	Above 35 °C

Sample Application in HPLC:

- Purity profiling
- Profiling or fingerprinting

- Method development
- Identification
- Quantification

Purity Profiling:

Purity profiling is a method of checking purity of a given standard.

Identification:

Identification is identifying the marker in the sample using particular standard.

Quantification:

Quantification is the process of estimating quantities of markers, APIs or other standards in plant extracts.

Applications of HPLC:

Pharmaceuticals

- Assay
- Related substances
- Stability Studies
- Compound identification
- Analytical method validation

Foods

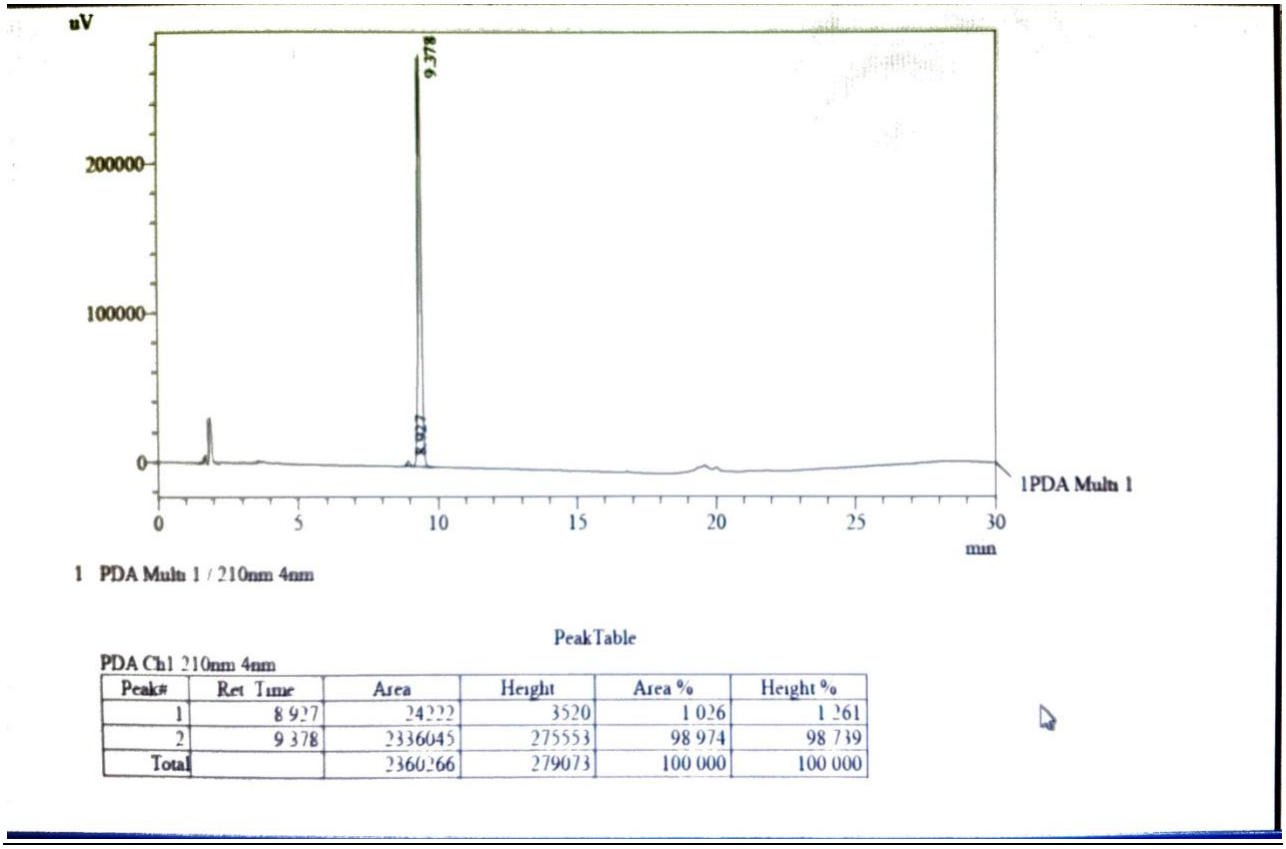
Separation identification and quantification of Fat-soluble vitamin, Water soluble vitamin, Amino acids, Aflatoxins, Steroids, Antioxidants and Dyes and synthetic colour.

Plant metabolites

Separation, Identification, quantification and isolation of secondary plants metabolites from plant extracts.

EXPERIMENT No. 1

AIM: PURITY PROFILING OF SAMPLE A



PeakTable

PDA Ch1 210nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.927	24222	3520	1.026	1.261
2	9.378	2336045	275553	98.974	98.739
Total		2360266	279073	100.000	100.000

Chromatographic Conditions

- Weight of sample A:
- Mobile phase: MPA – Acetonitrile (80%) water (20%) (0.1% H_3PO_4 in water)
- Wavelength: 254 nm, 250 nm
- Flow rate: 1.5 ml per minute
- Injection volume: 10 µl
- Vial no: 2
- Column oven temp: 40 °C
- Max pressure: 5000 psi Run time: 15 minutes
- Flow rate: 1.5 ml per minute
- Maximum pressure: 5000 psi

- Elution mode: Isocratic
- Column oven temperature: 40 ºC
- Column – ACE –Excel 5 C18 PFP, 250 mm x 4.6 mm x 5 µm
- Wavelength: 273 nm
- Injection volume: 10 µl

EXPERIMENT No.2

AIM: Calibration of HPLC Calibration of HPLC:

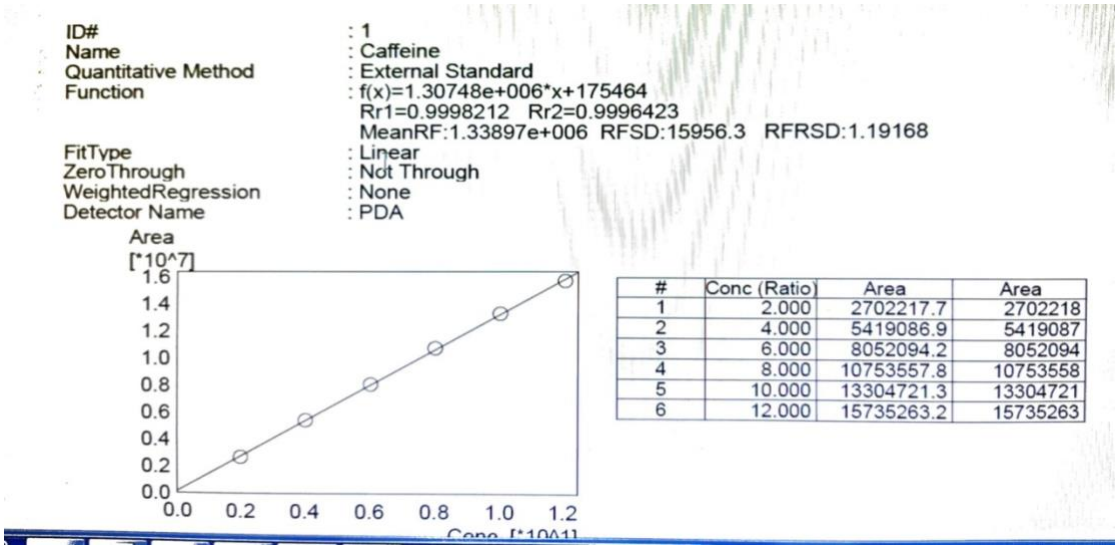
- Weight of sample= 1.78 mg, dissolved in 1 ml of methanol
- 1 mg/ml= 1000 ppm
- 1000 ppm: Standard stock solution
- 500 ppm: Standard solution
- Sample kept in vial no. 10
- Blank kept in vial no. 1
- Mobile phase A: Water 10%
- Mobile phase B: Acetonitrile 20%

Chromatographic Conditions:

- Run time: 15 minutes
- Flow rate: 0.8 ml/min
- Maximum pressure: 5000 psi
- Elution mode: Isocratic
- Column oven temperature: 40 ºC
- Column: YMC C18, 250 x 4.6 mm x 5 µm
- Wavelength: 273 nm
- Injection volume: 5 µL

Linearity:

Injection Volume	Area of the peak
2	2702218
4	5419087
6	8052094
8	5419087



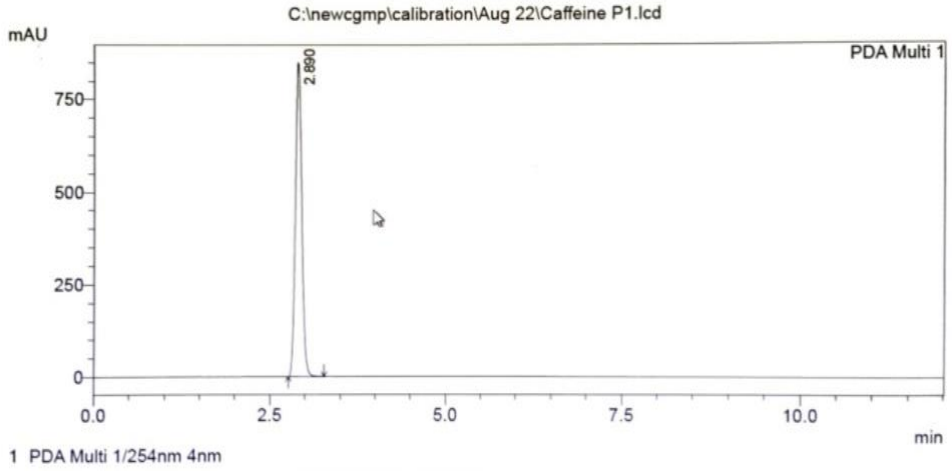
#	Conc (Ratio)	Area	Area
1	2.000	2702217.7	2702218
2	4.000	5419086.9	5419087
3	6.000	8052094.2	8052094
4	8.000	10753557.8	10753558
5	10.000	13304721.3	13304721
6	12.000	15735263.2	15735263

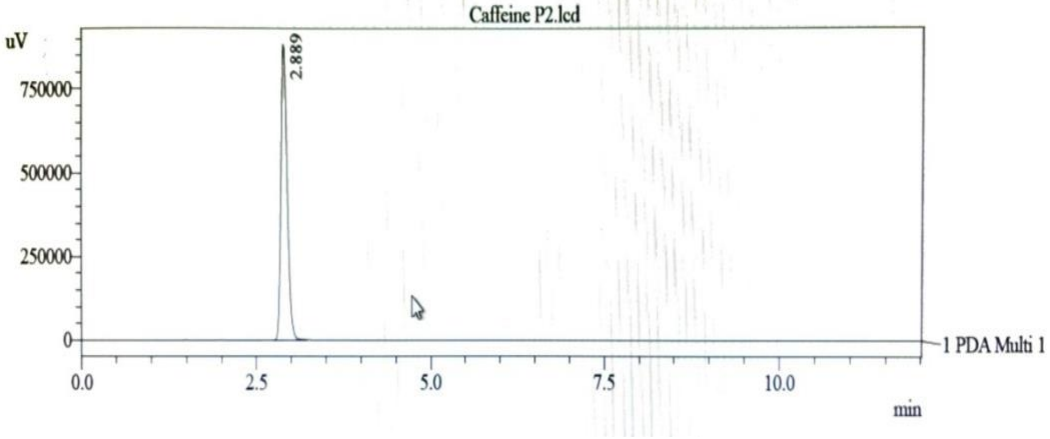
Precision:

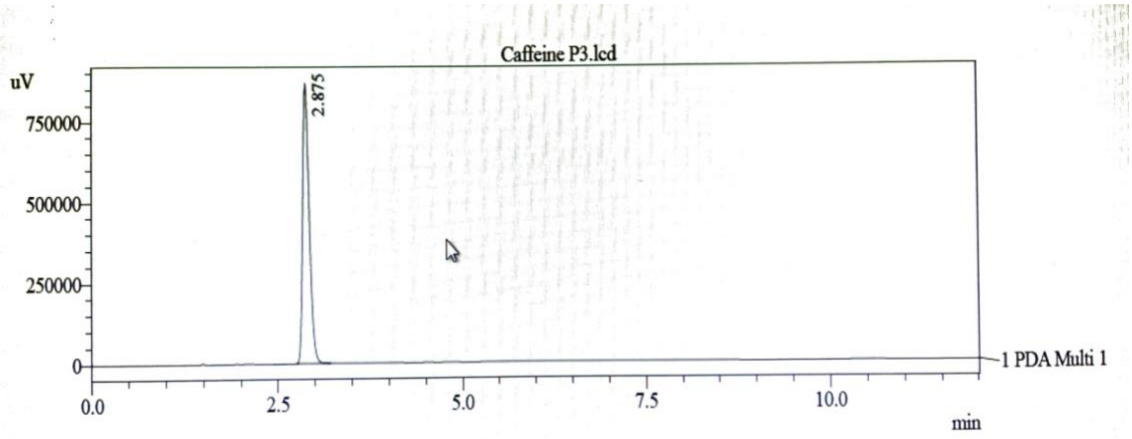
==== Shimadzu LCsolution Analysis Report ====

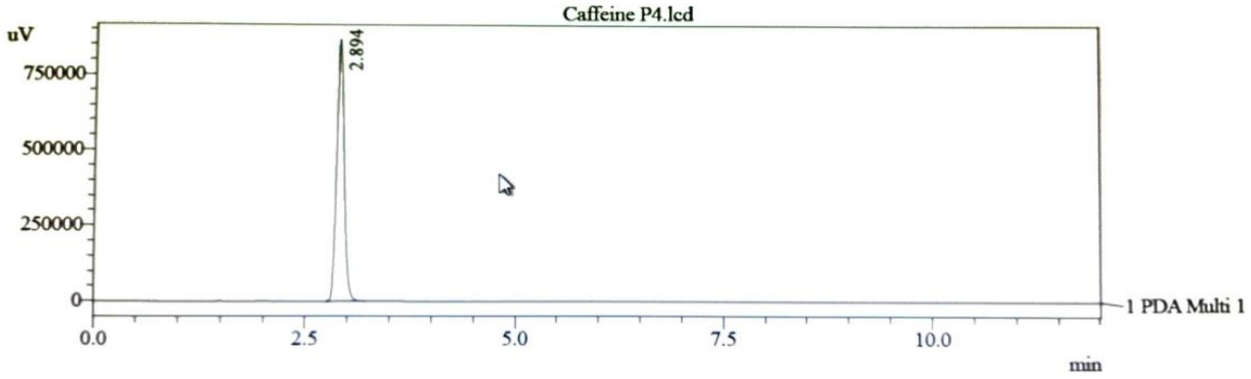
C:\newcgmp\calibration\Aug 22\Caffeine P1.lcd

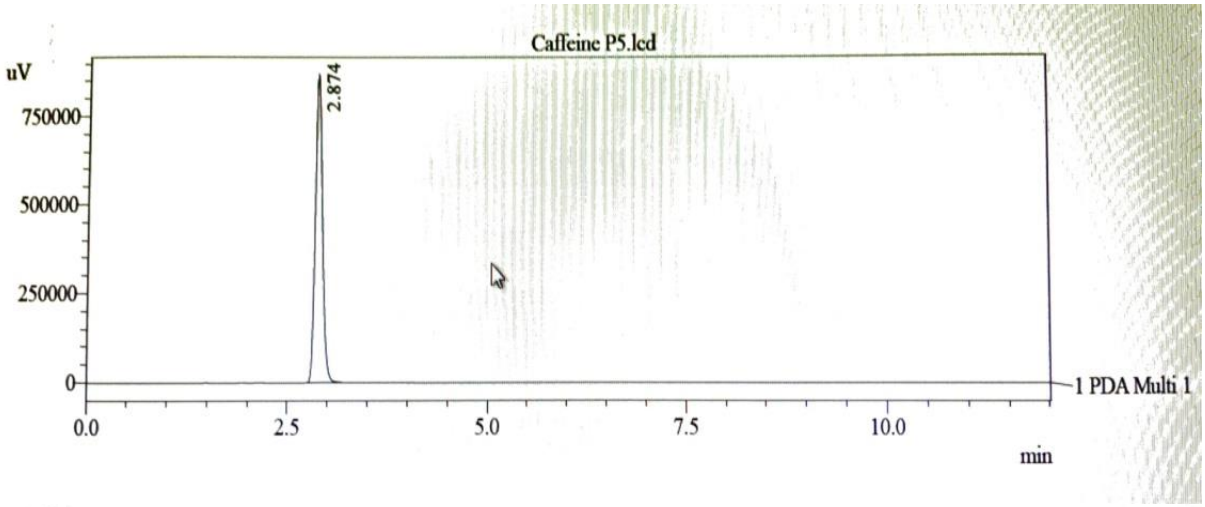
Acquired by	: Admin
Sample Name	: Caffeine P1
Sample ID	: Caffeine P1
Tray#	: 1
Vail #	: 9
Injection Volume	: 2 uL
Data File Name	: Caffeine P1.lcd
Method File Name	: CAFFINE CAL 26 August.lcm
Batch File Name	: 26082022.lcb
Report File Name	: Default.lcr
Data Acquired	: 30-08-2022 15:40:51
Data Processed	: 31-08-2022 15:11:44

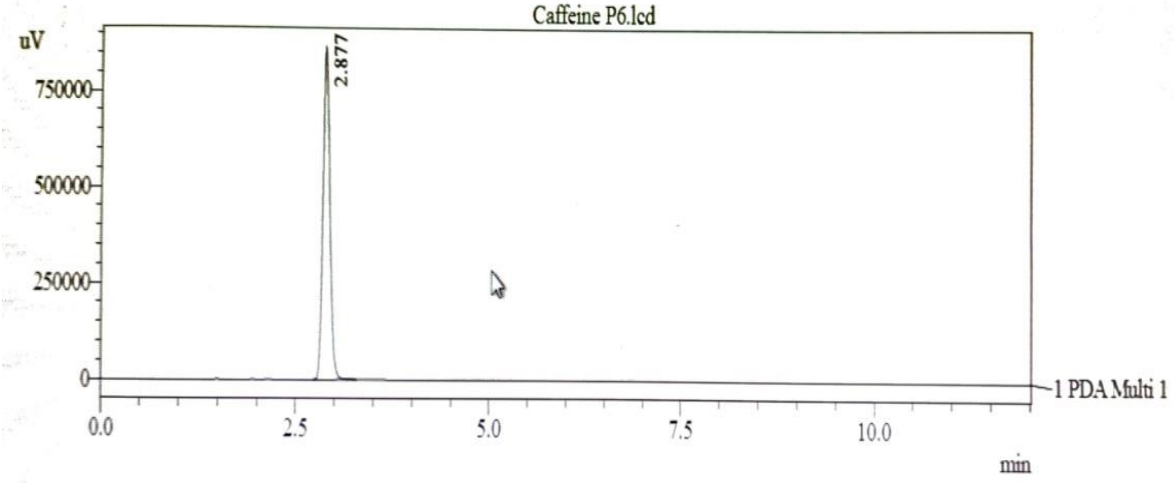

C:\newcgmp\calibration\Aug 22\Caffeine P1.lcd
mAU
PDA Multi 1
2.890
750
500
250
0
0.0
2.5
5.0
7.5
10.0
min
1 PDA Multi 1/254nm 4nm


Caffeine P2.lcd
uV
2.889
750000
500000
250000
0
1 PDA Multi 1
0.0
2.5
5.0
7.5
10.0
min


Caffeine P3.lcd
uV
2.875
750000
500000
250000
0
1 PDA Multi 1
0.0
2.5
5.0
7.5
10.0
min


Caffeine P4.lcd
uV
750000
500000
250000
0
2.894
0.0
2.5
5.0
7.5
10.0
min
1 PDA Multi 1

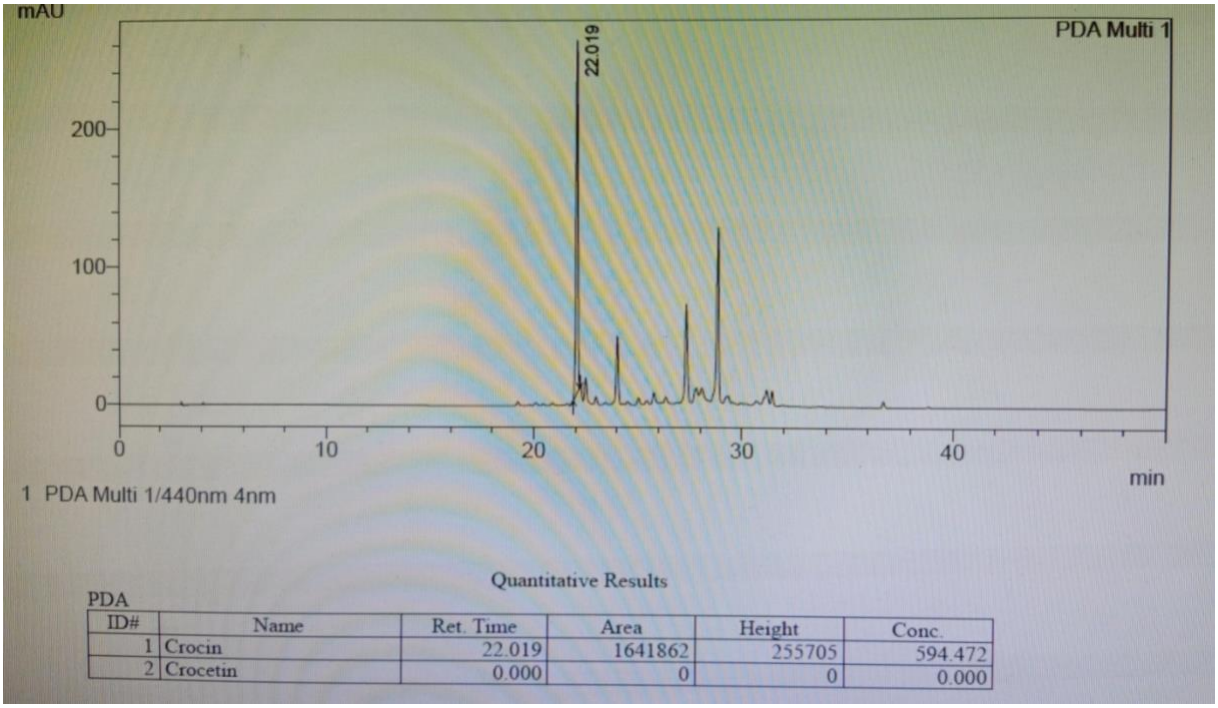

Caffeine P5.lcd
uV
750000
500000
250000
0
2.874
0.0
2.5
5.0
7.5
10.0
min
1 PDA Multi 1


Caffeine P6.lcd
uV
750000
500000
250000
0
2.877
0.0
2.5
5.0
7.5
10.0
min
1 PDA Multi 1

Experiment No. 3

Sample B:

Identification of marker in plant extract using standard:



Quantitative Results

PDA

ID#	Name	Ret. Time	Area	Height	Conc.
1	Crocin	22.019	1641862	255705	594.472
2	Crocetin	0.000	0	0	0.000

Chromatographic Conditions:

- Weight of standard: 1.1 mg
- Mobile phase A: 0.1% formic acid in water
- Mobile phase B: Acetonitrile
- Column: 250 x 4.6 mm x 5 µm- C18
- Flow rate: 1.0 mm/min
- Column temperature: 40 °C
- Run time: 50 mins
- Injection volume: 10 µL
- Wavelength: 440 nm

Gradient Elution Program:

Time (min)	Mobile Phase B
0.01	10
10	20
20	35
40	90
45	10
50	Stop

Conclusion

HPLC is high performance liquid chromatography which is also known as High Performance Liquid Chromatography. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture.

Applications in pharmaceuticals by the use of HPLC are Assay, related substances, stability substances, compound identification and analytical method validation, whereas in foods it is used for the separation identification and quantification of fat-soluble vitamin, water soluble vitamin, amino acids, aflatoxins, steroids, antioxidants, dyes and synthetic color.

HPLC finds it immense use in separation, identification, quantification and isolation of secondary plant metabolites from the plant extracts and all industries which deal with organic compound and with the help of HPLC one can isolate extremely pure compounds. It can be used used in both laboratory and clinical science.